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Editorial

The ethical obligations to the nonsubject

The problem of the obligations of the experimenter to his human subject is generally recognized as a highly charged one. No one will be surprised to find the issues explored in a symposium published in this JOURNAL. It seems to me, however, that the forgotten man is the innocent bystander, the patient whose medication will depend on the outcome of experiments on other men, the nonsubject whose apparent nonparticipation is only temporary since he will ultimately participate by virtue of the education he is given. Eventually, collectively, the nonsubject is an interested party, albeit a late comer. It is he who, sooner or later, will suffer or benefit from experiments or from the failure to perform the right ones. Quite seriously therefore it is proper to ask, "What are our responsibilities to the nonsubject—the real object of our experiments?"

We have a responsibility to the nonsubject when we publish because, regardless of where it appears, a hopeful statement on the usefulness of a drug acquires authority by virtue of its publication. The weight it carries is especially formidable when the statement is printed in a venerable journal, but regardless of where it appears printing establishes it, and what is more, once it is published the fact of publication is used to make the impression even deeper.

This accounts in part for the tendency to use drugs long after claims made for them have been proved meretricious. Even when a medicament can be replaced by a more effective one there is a general reluctance to alter established practice. Modest superiority is utterly incompetent and can produce no forward movement in the face of this inertia. Thus, along with our "miracle" drugs, modern medical practice continues to use a large number of useless and possibly even suspect therapies. The initial impetus supplied by poorly authenticated but published claims is not easy to overcome, and unsound therapies continue long as a part of accepted practice, to be dislodged only by something strikingly superior. Because of this, every insubstantial statement about a new drug or therapy that is published provides a threat to good medical practice and is a serious menace to progress. It therefore concerns all interested in medicine; it concerns the patient, it concerns the practicing physician, it concerns those who evaluate new drugs and therapies, and it is a pressing concern of this JOURNAL.

Superior experimental design is a critical

as well as a practical matter. Without well-conducted explorations in man as its basis accepted treatment may well be suspect. Because established practice is so often used as the basis of comparison with the new, if an established therapy which is to some degree harmful is compared with an ineffective therapy, the latter will appear to be effective. And from this it follows that, as long as a therapy being assessed is less harmful than the established therapy with which it is being compared, regardless of whether or not it hurts, it will inevitably appear to be beneficial. Only good or well-designed and well-conducted experiments can yield substantial information; even accidental discoveries turn out to be based on naturally or accidentally well-designed experiments.

The ethical question inevitably arises for those who evaluate new therapies whether one can justify an experiment with a suffering patient. But in view of the road block to medical progress thrown up by unsound claims, there should be a corresponding ethic which compels those who pursue investigations which are likely to establish therapies to use the best experimental design and to base conclusions on the soundest evidence and the most rigorous logic. If a statement on the usefulness of a particular treatment is true, why should it not also be statistically demonstrable? Why should a sound conclusion of an experiment in man require an emotional or a verbal prop for support? What possible defense can there be for not using the best methods in assessing the value of a drug to be used in man as well as in using man for assessing drugs? Nothing is too good for the experiment on man to be used for man. Nothing could be more unethical than a sloppy experiment on a human subject.

The desire to investigate is not only commendable but, since he emerged from the witch-doctor stage, has been a traditional preoccupation of the physician. Many are now needed to pursue this interest in order to cope with the spate of new drugs. But if the physician-experimenter takes his ethical responsibilities seriously he will not think that there are any easy "bits" of research for him to perform. He will not only follow the rules laid down for the selection of human subjects but, in due consideration for the objects as well as the subjects of his investigation, he will endeavor to make every procedure count, he will squeeze every last bit of information out of every experimental gambit. He will therefore use the most economic method, the method most likely to lead to sound, useful, and meaningful results, viz., the best experimental design.

Perforce, experimental design becomes an outstanding issue. Its importance extends far beyond an obligation to the subject of the experiment; its effect extends to those who may be led into using a drug that is really useless in preference to an effective one and those who may suffer if the drug is harmful. And there are also those who may not receive a useful new drug simply because the method of investigation used was not designed so as to reveal its special qualities.

It seems unlikely that pharmaceutical manufacturers would permit a useful drug to escape detection these days, but poor experimental design is just as likely to fail to reveal drug effects as it is to lead to the conclusions that ineffective drugs are useful. Many methods of investigation which seem to include every necessary control are not appropriate for the investigation at hand because they cannot sense the effects sought. While the features of clinical evaluation which are essential for the control of bias, such as randomization and double-blindness, have received such clear acceptance in many circles that these are often included in the titles of papers (e.g., A Controlled Clinical Trial of . . . or A Double-Blind Study of . . .), as if their use constituted a guarantee that the results were bound to be beyond reproach, and other features in experimental design in man are also well described in the literature, it often escapes notice that in particular circumstances a method with all these

features may nevertheless be totally blind. Insensitive or "all-blind" methods of clinical evaluation can be as misleading as methods which provide a positive result for every agent tested because, depending on what is compared with what, an insensitive method will tend to indicate that a useless drug is useful or that a harmful drug is harmless. In a recent study we have shown that, depending on how we chose our controls, with the same data we could demonstrate statistically that a well-known sedative drug either increased or decreased tremor.

Statistical validation is not an unchallengeable seal of approval. Statistics should be used only where it is useful, for an analysis of the data collected. It is the actual data, not the statistical validation, which prove the point, and no amount of statistical analysis can clarify an issue clouded by a poor experiment or muddied by contaminated data. At best, statistical analysis merely indicates the probability that differences which have been noted are not accidents of chance. When a difference has not been noted, statistical analysis very rarely indicates whether equality does in fact exist. Statistical analysis does not determine whether the data are good, bad, or indifferent, relevant or irrelevant to the issues involved, or, for that matter, worthy or unworthy of statistical analysis. In no case do they substitute for a searching analysis of the method used to collect them and of the logic behind the conclusions drawn from the collected data. The refusal of some to consider the need for statistical analysis of data in an experiment is no worse a crime than the willingness of others to swallow statistically approved conclusions without an examination of the quality of the data analyzed and of the method as a whole.

Many methods of clinical evaluation lack an indicator that the method can discern what it proposes to discover. How is one to know that a negative result truly indicates that a drug is ineffective unless it is also shown that the method is sufficiently sensitive to detect the effect in question? The chemist examines this issue when he determines the sensitivity of his balance and the discriminating powers of his methods of analysis. Clinical evaluation must show the same concern over the acuity of its methods. The ability to detect the difference between placebo and drug effects and the increments in effect which a method can distinguish must be demonstrated if a negative result is to be significant, if a positive result is to have quantitative meaning, if useful drugs are not to be overlooked, if in a comparison between them a harmful drug is not to be reported as not different from a useful drug, if human subjects are not to undergo a useless experimentation, and human patients are not to be given useless or even harmful new medicaments, or to be chained to inferior old ones.

It was stated by Topley that once a treatment was accepted as orthodox the organization of a controlled clinical trial became almost impossible. This is a serious matter because, even if ineffective old therapies do not hurt a patient by a direct action, they may injure him immediately enough by keeping from him more effective treatment and they may seriously harm him in the end by getting in the way of progress in therapeutics. In view of the plethora of new drugs this is of crucial importance to all involved for unless these issues are effectively met, in the not too distant future we may find progress completely paralyzed by the many false standards set up by unsound investigations.

The essential experiments in clinical evaluations in man consume much time and effort at a critical juncture in the history of pharmacotherapeutics when we already have about as many new and active drugs as we can possibly evaluate properly. And since nothing gets in the way of the development of better therapies so much as poorer ones that are generally accepted, the untoward effects, the side effects, the toxic effects of investigations of poor quality far exceed in significance anything that may have to be sacrificed in waiting for

the proper performance of a drug assessment. This places a serious responsibility on those who do clinical experimentation and on all who have anything to do with the publication of papers in medical journals, editors and publishers as well as authors. It is certainly our plan to accept this responsibility seriously; it is a big part of a reason for being.

Walter Modell, M.D.

A hypothesis is a proposition which seems to explain observed facts and whose truth is assumed tentatively for purposes of investigation. It is a leading question put to nature, a guess designed to suggest the sort of inquiry by which an answer might be reached. The success of the method of hypothesis depends upon the delicate balance between conjectural and empirical elements. We have to construct guesses as to how thi[ng]s before we can make the observations necessary to determine whether our gue[s]s[es] [a]re correct. The hypothesis, then, is a proposition suspected of being tr[ue] [b]ut lacking the requisite support of evidence, a suggestion that new a[nd] [n]ecessary relations may exist and should be investigated. A good hypothesis is o[ne] that offers a possible explanation or that ascribes an adequate cause.

In other words, a hypothesis is a preconception of what investigation will disclose. It must be a matter about which there is both doubt and some inclination toward belief. Where there is no doubt concerning the truth of the idea, there is no willingness to submit it to the test of experiment. No one would try a proposition already convicted on conclusive evidence, and no one would test a proposition whose truth is not doubted. Without the inclination to belief, there would be no disposition to look into the degree of truth of the hypothesis. For if the hypothesis should be confirmed, it could be used as an instrument in further inquiry. And without the large element of doubt, the proposition would be a simple matter of belief, not a scientific hypothesis.

From "The Role of Hypotheses in the Scientific Method," by James K. Feibleman, Perspectives in Biology and Medicine, Volume II, Number 3, Spring, 1959, by Permission of the University of Chicago Press.

Commentary

The nature of the antirheumatic action of salicylates

The problem of the mode of antirheumatic action of salicylates is approached through a consideration of structure-activity relationships. Experimental properties of salicylates and structurally related compounds which parallel antirheumatic properties include the ability to produce elevated circulating corticoid levels, acidic strength (dissociability), chelating potential, and the ability to suppress experimental arthritis. Effects which do not parallel antirheumatic properties include uncoupling of oxidative phosphorylation, stimulation of oxygen consumption, effects on carbohydrate metabolism, and inhibitory effects on immunologic processes. The possible interrelationships of the various experimental effects and the application of studies on structure-activity relationships to the problem of the mode of acton of salicylates are discussed.

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Despite the fact that the antirheumatic effects of salicylates have been known for over eight decades, the mode of action of this group of drugs remains obscure. The effects of salicylates on the acute symptomatology of rheumatic fever are so dramatic as to lead almost implacably to the conclusion that they cannot be accounted for on the basis of analgesic and antipyretic properties alone. This has resulted in numerous attempts to delineate the mode of antirheumatic action of salicylates.

An important (and often neglected) aspect of studies on the mode of action of a drug is a consideration of structure-activity relationships. Ideally, an experimental effect of salicylates, if it is to be acceptable as a potential explanation for their antirheumatic properties, should not be reproduced by therapeutically inactive structural analogs. By the same token, effects similar to those produced by salicylate should be expected from all structural analogs that do have antirheumatic properties. There are, however, certain limitations which must be taken into account in interpreting the results of structure-activity studies, particularly where in vitro experiments are involved. A compound which is inactive in vitro may be converted in vivo to an active metabolite. Similarly, a compound may be active in vitro but circulate in vivo only in an altered form which may or may not be active. Problems of distribution in the tissues may also prevent an accurate extrapolation from the in vitro to the in vivo situation. Thus, in the living organism the drug may fail to reach the very system which is being affected in vitro. Finally, there is the problem of relative concentrations: an effect should be produced with concentrations of the drug of

Table I. *Comparative experimental effects of salicylate and structurally related compounds in relation to antirheumatic properties*

Compound	*Corticoid effect**	*Uncoupling†*	*Stimulation O_2 consumption‡*	*Depletion liver glycogen§*	*Mucopolysaccharide effect‖*	*Fibrinolysin inhibition¶*	*vs. antigen-antibody precip.***	*Inhib. exper. arteritis††*	*Antianaphylaxis‡‡*	*vs. localized Schwartzman §§*	*vs. anaphylactic arthritis‖‖*	*pK_a ¶¶*
*Antirheumatic****												
Na salicylate		+			+	+	+					3.0
	+		+	+				+	+	+	+	
Aspirin		(+)			(+)	(−)	(+)					
Gentisate	+	−	−	±	−	−		−	−	−	+	3.0
γ-resorcylate	+	−	−	−		−		−			+	2.7
3 OH salicylate	+	+	−									3.1
4 OH salicylate	+	−	−	−	−					−		4.7
*Not antirheumatic****												
Benzoate	−	−	−		±	−					−	5.8
m–OH benzoate	±	−	−	+	−	−	+	−			−	5.9
p–OH benzoate	−	−	−		−	−	+	−	−		−	5.5
Salicylurate	−	−				−					−	

+=Active;—=inactive; blank=not known.
*Production of elevated plasma corticoid levels in guinea pigs.[7]
†In vitro uncoupling of oxidative phosphorylation.[8,9]
‡Stimulation of oxygen consumption of rats.[10]
§In vivo depletion of liver glycogen in rats.[11]
‖Inhibition of sulfate exchange of chondroitin sulfuric acid in slices of calf cartilage in vitro.[12]
¶In vitro inhibition of fibrinolysin.[13]
**Inhibition of precipitation of antiprotein and antipolysaccharide antigen-antibody system in vitro.[14]
††Prevention of arteritis induced in sensitized rabbits by injection of bovine gamma globulin.[15]
‡‡Prevention of death from anaphylaxis induced in sensitized rabbits by egg albumin.[16]
§§Inhibition of localized "Schwartzman phenomenon" in rabbits.[17]
‖‖Inhibition of passive Arthus phenomenon produced in guinea pigs by the intra-articular injection of egg albumin following the intracardial administration of anti-egg albumin rabbit serum.[13]
¶¶$pK_a = -\log K$ (dissociation constant). Reflection of chelating ability, low pK_a values being related to chelating capacity.[18,19]
***From the data of Stockman[20]; Clarke, Clarke, and Mosher[21]; and scattered reports in the literature.

the order of that obtained in the blood during therapy if it is to be acceptable as a possible explanation for the mode of action.

If done carefully and with due regard for limitations, a consideration of the structure-activity spectrum may be helpful in evaluating the potential contribution of various studies to an understanding of the mode of antirheumatic action of salicylates. It may also provide a basis for speculation on possible interrelationships among the various experimental effects produced by these substances. Table I presents a comparison of certain experimental effects of salicylates and structurally related compounds; it will be discussed with the full realization that much pertinent information relative to metabolic products and tissue distribution is not available. Since aspirin is converted principally to salicylic acid in vivo, the properties of these two compounds are considered together; however, the effects of aspirin in the in vitro experiments are shown separately in parentheses. The structures of the various compounds are shown in Fig. 1.

Effects on pituitary-adrenal system. The demonstration of the antirheumatic properties of adrenocortical hormones and adrenocorticotropin (ACTH) led to considerable speculation that the antirheumatic effects of salicylates may be mediated through the

hypothalamic-pituitary-adrenal system. Numerous studies have demonstrated striking similarities between the metabolic and anti-inflammatory effects of salicylates and adrenocortical hormones or ACTH, while other studies have indicated that salicylates and corticoids have some effects which are actually opposing. These have been well reviewed elsewhere[1-6] and will not be enumerated here. For the most part, the effects of salicylates on carbohydrate metabolism are the reverse of those produced by adrenocortical hormones. Notable in this regard are (1) the reduction in hyperglycemia and glycosuria which is produced by salicylates in diabetic human subjects or experimental animals and (2) the depletion of liver glycogen by salicylate in experimental animals. Salicylates are known to have striking effects of their own on intermediary metabolism which are distinct from any possible effect on the adrenal cortex since they can be produced in vitro. For this reason, if salicylates stimulated the pituitary-adrenal system, it would not be surprising to find that the effects on carbohydrate metabolism did not parallel those produced by corticoids. Their failure to do so does not seem to constitute a potent argument against the concept that the antirheumatic action of salicylate is mediated, at least in part, through the pituitary-adrenal system.

Considerable confusion has arisen from attempts to determine directly whether salicylates stimulate the pituitary-adrenal system. Much of the prevalent confusion, particularly with regard to structure-activity relationships, has arisen from studies in which such nonspecific indices of adrenocortical function as the responses of adrenal ascorbic acid or circulating eosinophils were used. Unfortunately, the problem has not been clarified completely by

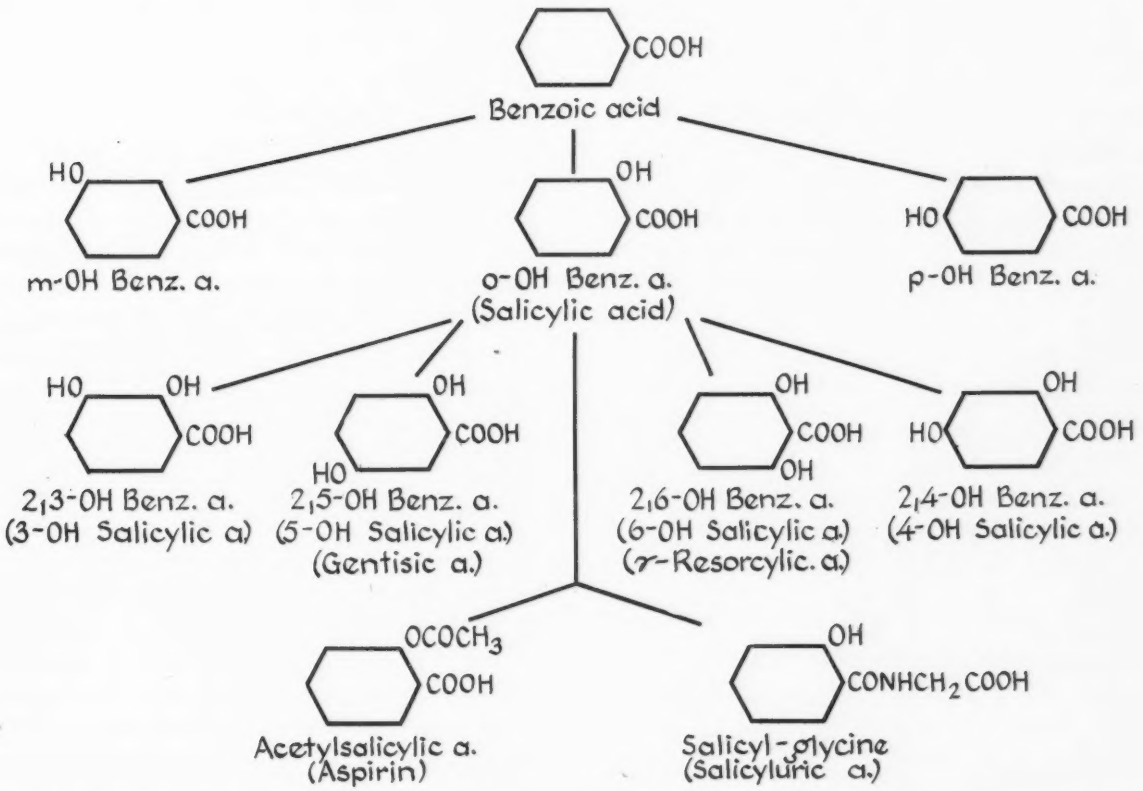


Fig. 1. Structures of salicylates and chemically related compounds. (Salicyluric acid is the principal metabolite of salicylate.)

studies in which measurements of adrenocortical hormones in blood and urine were performed. It seems clear that elevated blood levels of corticoids occur in otherwise normal human subjects with salicylate intoxication and in experimental animals given relatively large doses of salicylate. It is not known, however, whether this represents anything more than a nonspecific "stress" response. The propensity for producing striking elevations of circulating corticoid concentrations appears to be restricted to compounds having antirheumatic properties (Table I). Less clear are the effects of *therapeutic* doses of salicylates and structurally related antirheumatic compounds on the production and disposition of corticoids. Most workers have failed to find clear-cut elevations of circulating corticoid concentrations in normal or rheumatic human subjects given therapeutic doses of salicylate. The urinary excretion of various corticoids has been found to be increased, decreased, or unaffected. Discrepancies among the findings of various investigators may be due to differences in doses of salicylates employed, the subjects used, and the methods employed for steroid measurements. It is certain that if the antirheumatic effects of salicylates are in any way dependent upon intervention of the pituitary-adrenal system, they are not dependent upon the *maintenance* of elevated circulating levels of corticoids. The possibility that salicylate simultaneously affects the production and disposition of adrenal hormones[3] seems deserving of further investigation. In any event, in our present state of knowledge, dismissal of the possibility that the antirheumatic effect of salicylates is mediated through the pituitary-adrenal system or its secretions seems premature.

Metabolic effects. A number of workers have shown that salicylate increases oxygen consumption and uncouples oxidative phosphorylation.[8-10,22-24] The possibility has been considered[25] that this may be the basis for the anti-inflammatory activity of salicylates and other nonhormonal antirheumatic drugs. The similarity of salicylate and the classical uncoupling agent, 2,4-dinitrophenol, in regard to effects on oxygen consumption and oxidative phosphorylation have been noted. On the other hand, recent studies[26,27] have shown that there are distinct differences in the effects of salicylate and 2,4-dinitrophenol. New techniques for the study of oxidative phosphorylation have led to the discovery that different uncoupling agents (e.g., dinitrophenol and thyroxine) may differ in mechanism of action.[28] Thus, differences in the over-all effects of dinitrophenol and salicylate might be explainable on the basis of differences in the mechanism and energetic consequences of the uncoupling process. The relationships among structure, antirheumatic properties, and the ability to produce uncoupling of oxidative phosphorylation and stimulation of oxygen consumption are summarized in Table I. While these effects are not produced by compounds which are devoid of antirheumatic properties, they likewise are not produced by many of the structural analogs of salicylate which are antirheumatic. Particularly notable in this regard is γ-resorcylate which does not interfere with oxidative phosphorylation, but has a markedly enhanced antirheumatic potency by comparison with salicylate.[29] It is worthy of note also that 2,4-dinitrophenol has no effect upon certain experimental inflammations which are inhibited by salicylates.[25] These data suggest that the antirheumatic properties of salicylates and related compounds are not related to stimulation of oxygen consumption or uncoupling of oxidative phosphorylation. They also suggest that there is no relationship between stimulation of oxygen consumption or uncoupling of oxidative phosphorylation and the effect of salicylates on the pituitary-adrenal system. However, these interpretations are subject to the previously mentioned limitations inherent in in vitro structure-activity studies.

While there is little reason for suspecting that the effects of salicylate on carbohydrate metabolism and on the rheumatic

process are causally related, it is possible that there is a common denominator for these and other effects. A major effect of salicylate on carbohydrate metabolism is depletion of liver glycogen.[11,30,31] It can be seen in Table I that depletion of liver glycogen in the intact rat is produced by m-hydroxybenzoate, which has no antirheumatic properties, and is not produced by γ-resorcylate or 4-hydroxysalicylate, each of which is antirheumatic. These findings suggest that the antirheumatic effects of salicylates are not correlated with the effects on carbohydrate metabolism as reflected by liver glycogen. The data suggest also the lack of a common denominator for effects on carbohydrate metabolism and circulating corticoid levels. Glucocorticoids and salicylate have opposing effects on liver glycogen, the former causing glycogen deposition which is prevented by the simultaneous administration of salicylate.[31] The structure-activity spectra of the uncoupling and the glycogen effects differ somewhat, but difficulties of extrapolating from the in vitro to the in vivo situation prevent the formulation of definitive conclusions regarding their possible interrelationships.

Effects on connective tissue metabolism. The action of salicylates and related compounds on the sulfate exchange of chondroitin sulfuric acid has been studied on the theory that drugs useful as antirheumatic agents might influence the metabolism of certain of the chemical constituents of mesenchymal tissues.[12] Cortisone is known to decrease the sulfate exchange of chondroitin sulfuric acid. It can be seen in Table I that the sulfate exchange of chondroitin sulfuric acid in slices of calf cartilage in vitro was inhibited by sodium salicylate and aspirin, but not by the two additional antirheumatic structural analogs which were studied (gentisate and 4-hydroxysalicylate). In addition, slight inhibition was obtained with benzoate. These observations suggest that sodium salicylate and aspirin share with cortisone the ability to inhibit the synthesis and metabolic activity of mucopolysaccharides in mesenchymal tissues, but the fact that similar effects are not produced by antirheumatic compounds structurally related to salicylate detracts from the acceptability of this phenomenon as an explanation for the antirheumatic effects of salicylates and related compounds.

Fibrinolysin inhibition. There is considerable evidence that the proteolytic enzyme fibrinolysin may play an important role in the development of inflammation. For this reason, the possibility that anti-inflammatory substances may inhibit fibrinolysin has been investigated.[13] Sodium salicylate, antipyrine, aminopyrine, 3-hydroxy-2-phenylcinchoninic acid (HPC) and p-aminophenol, each of which inhibits experimental inflammation, have been found to inhibit fibrinolysin in vitro. As can be seen in Table I, however, this property is not shared by gentisate or γ-resorcylate, both of which are antirheumatic. Thus, there is not a convincing parallelism between this in vitro effect and antirheumatic properties. On the other hand, there is not sufficient information on the metabolic fate of the discrepant compounds to rule out completely the possibility of a relationship between fibrinolysin inhibition and antirheumatic effects.

Effects on immunologic processes. Sodium salicylate and aspirin inhibit the precipitation of both a typical antiprotein and antipolysaccharide antigen-antibody system, presumably due to an increase in the solubility of the antigen-antibody complex.[14] Structurally related compounds which possess antirheumatic properties were not studied. However, the fact that similar effects were produced by the therapeutically inactive meta- and para-hydroxy derivatives of benzoic acid (see Table I) suggests that there is no relationship between this experimental phenomenon and clinical antirheumatic effects.

Sodium salicylate and aspirin have been found to inhibit the production of coronary arteritis in sensitized rabbits injected with bovine gamma globulin.[15] Similar protection is afforded by cortisone. Protection was

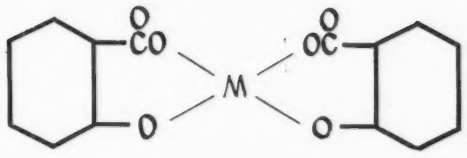
not afforded, however, by gentisate or γ-resorcylate, both of which have antirheumatic properties (Table I). Fatal anaphylaxis induced in sensitized rabbits by the injection of egg albumin is inhibited by aspirin, sodium salicylate, and aminopyrine, but not by gentisate.[16] The phenomenon of local necrobiosis and vascular damage elicited in rabbits by combined local preparatory and intravenous provocative injections of certain bacterial filtrates (the "localized Schwartzman phenomenon") is inhibited by ACTH, cortisone, aspirin, sodium salicylate, and HPC, but not by gentisate, 4-hydroxysalicylate, phenyl salicylate, acetanilide, or aminopyrine.[17] These findings offer evidence against the concept that the antirheumatic effects of salicylates and related compounds are mediated *directly* through an inhibitory effect on hypersensitivity. The data also suggest the lack of a relationship between salicylate inhibition of at least these particular hypersensitivity reactions and pituitary-adrenal stimulation.

In contrast to the studies described above, there is a striking structure-activity parallelism between antirheumatic properties and inhibition of anaphylactic arthritis[13] (Table I). In these studies, guinea pigs were given an intra-articular injection of egg albumin following the intracardial administration of anti–egg albumin rabbit serum; the effects of various drugs on the degree of joint swelling were used as the index of inhibition of this phenomenon. Inhibition was obtained with sodium salicylate, aspirin, gentisate, γ-resorcylate, antipyrine, aminopyrine, phenacetin, p-aminophenol, and HPC, but not with the therapeutically inactive structural analogs shown in Table I. It is not clear, however, whether the inhibition of joint swelling in these experiments is the result of an inhibitory effect of the active compounds on immunologic processes or simply reflects the known ability of salicylates to diminish joint permeability. (Salicylate suppresses the joint swelling produced by the intra-articular injection of a variety of irritant substances under circumstances which do not suggest the intervention of immunologic processes.) It is possible that these results reflect pituitary-adrenal stimulation since similar doses of the active drugs are known to produce elevated circulating corticoid levels in guinea pigs.[7] In addition, the structure-activity spectra of the two effects are similar.

Ionization and chelation. Among the hydroxylated derivatives of benzoic acid, those with a hydroxyl substitution in the ortho position have the highest dissociation constants (lowest pK_a values). Since these are also the compounds which are therapeutically active, there arises the possibility of an association between these two phenomena (Table I). The increased ionization of the ortho hydroxyl derivatives over other hydroxybenzoic acids has been attributed to chelation of the anion.[18] Among compounds capable of forming chelates, pK_a parallels the stability of chelates[19]; thus, in Table I pK_a can be considered to be an index of chelation potential: the lower the value for pK_a, the greater the ability to form stable chelates. It has been theorized[29] that the antirheumatic effects of salicylates and similar compounds are related to ability to chelate. Were this the case, one might expect that γ-resorcylate (2,6-dihydroxybenzoate), because of its two ortho hydroxyl groups, would have enhanced antirheumatic potency, since its ability to form stable chelates would be enhanced. This compound has, indeed, been found to have superior antirheumatic potency, although its therapeutic usefulness is limited by its increased toxicity. Fig. 2 illustrates the proposed structure of salicylate and γ-resorcylate chelates. The possibility of a relationship between chelation and antirheumatic properties remains open, although the mechanism whereby such a relationship could exist remains obscure. It has been suggested, though the evidence is not yet convincing, that chelation is of importance in the mechanism of action of glucogenic corticoids.[33]

The compounds which are capable of inducing elevated circulating corticoid levels have higher dissociation constants (lower

a.



b.

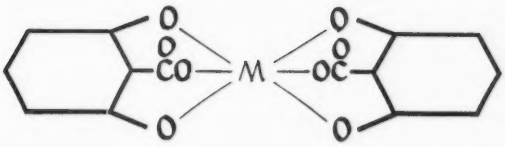


Fig. 2. Proposed mechanism of chelation of a metal M *(a)* by salicylic acid[19] and *(b)* by γ-resorcylic acid. Structure of the resorcylate-metal complex was hypothesized analogically on the basis of the structure proposed[22] for other chelating substances having one primary and two coordinating valences.

pK_a values) and presumably a greater ability to form chelates than compounds which do not affect corticoid levels. Obviously, a causal relationship has not been established; however, structure-activity considerations are consistent with the possibility that there is a causal relationship. On the other hand, the data suggest that there is no relationship between dissociability or chelating potential and uncoupling of oxidative phosphorylation, stimulation of oxygen consumption, depletion of liver glycogen, inhibition of fibrinolysin, or depression of immunologic phenomena.

Conclusions

It is apparent that the mechanism whereby salicylates and structurally related compounds exert their antirheumatic effects remains an enigma. None of the studies discussed here seems as yet to offer an entirely satisfactory explanation although this may come about through additional investigation of these possibilities.

When the effects of salicylates and structural analogs are considered in terms of structure-activity relationships, some appear to parallel antirheumatic properties while others do not, and parallelism among certain of the experimental effects themselves is noted. Of the effects discussed, the ability to produce elevated circulating corticoid levels, dissociability (which also reflects ability to form chelates), and the inhibition of anaphylactic arthritis (perhaps reflecting only diminished synovial permeability) correlate best on a structure-activity basis with antirheumatic properties. The possibility that these various effects have a common denominator or are causally related one to another or to suppression of inflammation deserves further investigation. It is possible that certain of the in vitro effects bear a closer relationship to antirheumatic activity than is suggested by structure-activity relationships. However, further evaluation of this possibility must await a better understanding of the metabolism and tissue distribution of the test compounds, and perhaps the development of techniques for studying analogous properties in vivo.

Attempts to elucidate the mode of action of antirheumatic substances are hampered by a lack of understanding both of the disease process itself and of the characteristics of the tissues which are affected. Further basic information is needed concerning the chemical and metabolic properties of connective tissue, the physical and chemical concomitants of inflammation, and the pathogenesis of the rheumatic process.

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Symposium on the study of drugs in man

Part II. Biologic and medical studies in human volunteer subjects; ethics and safeguards

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The study of man is an essential aspect of biologic research as it bears on the field of medicine. This fact arises because of the urgency of problems in clincal surgery and medicine, and the egregious species differences seen in the vertebrate world.

The beginning of scientific study of man as a biologic system is to be found in the anatomic dissections of Vesalius in Padua during the period 1535-1545. The growth that started there has never ceased nor flagged; its culmination represents the field of clinical investigation today. Vesalius suddenly realized, while lecturing in Bologna, that most previous knowledge of anatomy (and a large fraction of his own previous teaching) had been based on anatomic dissections carried out on pigs, dogs, and monkeys. This realization gave him a sense of shame and defeat and constituted the stimulus that was to result in the publication of the *Fabrica* five years later. In this ancient experience, 400 years ago, we may find many modern parallels and important lessons to be learned.

Over the intervening years the greatest share of the study of pathologic situations or abnormal settings in man has been carried out in conjunction with the clinical care of the sick. This type of research has come to be known as "clinical investigation," as opposed to "laboratory investigation," although there should properly be no divorce or dichotomy in the conceptual background of the two. It has nowhere been better epitomized than in Fuller Albright's presidential oration before the Society of Clinical Investigation in 1948. Here Dr. Albright spelled out clearly the many vagaries and pitfalls involved in the study of man, the necessary lack of control, and the multiplicity of variables. Nonetheless, he made it equally clear that the presence of disease processes often provides a beautiful opportunity to study special physiologic, anatomic, or functional deficits and that a careful quantitative approach to the problem at hand is ever the mark of the competent investigator, whether working in man, animals, or test tubes.

Of recent years, the study of controlled experimental situations in normal man has become increasingly important. Such studies have often been carried out as a part of teaching, and often in conjunction with the undergraduate medical school teaching of physiology and biochemistry. The

student, the subject, and the investigator are one and the same person. It seems quite an innocent matter to give the medical student a drink of glucose and then measure his blood and urine sugar, to plot a curve of renal threshold or glucose tolerance. It is a long step from this research "all in the family" to the use of the uninformed lay person, laying himself open to the possibility of personal injury, as a normal volunteer for medical study.

The multiplicity of problems that surround this latter situation has given rise to this symposium; in this paper I shall try to discuss a few of these problems. To indicate my own qualifications, I should state that over the past fifteen years we have studied normal man in order to gain a better understanding of the metabolic and biochemical changes associated with the posttraumatic state. Our studies of normal man have ranged from simple observational metabolic-balance techniques through starvation, immobilization, the use of endocrine substances, and general anesthesia. Our experience is not numerically large, yet we have learned many lessons from it.

I. Species differences

Species differences in the vertebrate world must be sharply differentiated as between those that relate to intracellular molecular and enzymatic processes on the one hand and those that involve whole-organ systems or integrated physiologic reactions on the other. There may be differences between the rat, the rabbit, the dog, and man with relation to the metabolic arrangements in the tricarboxylic acid cycle, in the conversion of prothrombin to thrombin, or in the amino-acid composition of immune globulins—as examples of phenomena at the cellular-molecular level—but if such differences exist, they have rarely been described and they do not seem to be important. If one is going to study the ability of the liver slice to oxidize carbon-labeled glucose, one can study many of the influences bearing on this reaction equally well in the liver of the mouse, rat, hamster, rabbit, cat, dog, monkey, or man—to mention but a few species.

When we turn to whole-organ systems and integrated physiologic reactions, we find an entirely different situation. The rat, for example, is an omniverous, constantly eating, night crawling, long-tailed rodent. The dog is a pronograde carnivore of the canine hunting type, given to chasing and securing its meat in violent physical conflict, often at some danger to itself, and eating infrequently when such prey is found, followed by a sleep in the sun. The muscle groups of the fore and hind limbs of the dogs are approximately equally balanced and the assumption of the upright posture requires a minimum of autonomic activity to redistribute the blood. The upright posture is maintained with little muscular activity, somewhat as in the case of a horse which can fall asleep standing up. The dog has a very excitable autonomic system, has special arrangements with respect to outflow from the portal circulation, and has a stomach capable of digesting the hair, fur, and skeleton of its prey. The cat is an arboreal night prowler, carnivorous like the dog but with a more highly developed hind-limb musculature necessary for the spring employed in seizing its food. Man is an erect walking, omniverous, constantly eating biped—who eats whenever he can find food, which is quite frequently, and eats multiple small meals, often digesting them quite satisfactorily with a minimum gastric digestive capacity and a gastrointestinal mucous membrane that is extremely sensitive to autodigestion. The erect posture has brought in its train two remarkable adjustments. One has been the location of a tremendous mass of lean potassium-containing, energy-exchanging tissue between the knee and the navel. The quadriceps, hamstrings, adductors, abductors, and glutei form a localized mass of muscle required to maintain the pelvic wheel in its upright rotation and whose function and upright posture require continuous neural and kinesthetic control for its maintenance. Wounds and injuries of this large muscle mass have no counterpart

in pronograde quadrupeds. The autonomic arrangements of man are finely tuned to the upright posture and tuned with such a fine edge of discrimination that even a few days in bed will abolish the postural vascular reflexes and require a day or two for their normal resumption.

These three species exhibit a number of other phenomena that contrast them sharply. The rat, for instance, is extremely sensitive to starvation. It is for this reason that a rat-infested community can be cleaned of rats merely by cleaning the community. The starved rats soon eats its cagemates, the fire of life burns low, and then it dies in eight or ten days. The dog, by sharp contrast, tolerates starvation extremely well and periods of a week of starvation are quite normal in the canine in the wild state, particularly in the winter. But man is the most resistant to starvation of these three. Starved man, after many weeks or months of drastic semistarvation, still maintains motivation and muscular activity adequate to escape from the enemy, to walk over desert or snowy wastes, to sail in a small boat great distances and achieve survival. Centuries of literature bear this out and it needs no special biologic confirmation.

Such points of species differences as between the rat, the dog, and man might seem small or unimportant but as soon as one starts to study any sort of deprivation state, or the reaction to trauma, injury, or shock, one finds that these three species have differences that are irreconcilable. It is not surprising that endocrine relations, nervous reactions, and bacterial processes cannot always be equated.

The hepatic outflow tract of the dog was mentioned above and deserves an additional word. In simple hemorrhagic shock a change occurs in the outflow tract of the canine liver that is best demonstrated by leaving the animal in shock for a while and then suddenly retransfusing his lost blood. In many but not all instances the observer will note three spectacular alterations within a few minutes. The liver becomes large, tense, purplish black, turgid, and immensely swollen. The pressure in the portal veins rises sharply to high levels. After an additional period the edematous, swollen mucosa of the gut starts to bleed. The anatomic, physiologic, biochemical, and bacteriologic aspects of this hepatic outflow obstruction in the dog have recently been studied by many workers and it will not be my purpose to review them here. The important point is that this characteristic canine sequence, which seems to be critical in survival of the dog after hemorrhage, has no parallel in man. Massive hemorrhage from the gut has never been reported as a complication of shock alone, in man. Thousands of men in shock—in World War II and Korea—have been subjected to the careful scrutiny of competent clinical investigators and this phenomenon has never been seen. As a surgeon, I—and many other surgeons interested in abdominal disease—have operated on many patients in shock or patients who have recently been in shock and who have then been transfused. One never sees a swollen, black, turgid liver, or portal hypertension in such cases.

This seemingly minor detail is mentioned here merely as an excellent example of an important species difference between the dog and man that makes the translation of data on experimental shock in the dog to clinical shock in the man extremely hazardous. This promising and important work has opened many new and important vistas, but its direct clinical application to man would be fallacious.

These, then, are some of the things that lurk in the background as the biologist views a normal human volunteer who might be willing to forego food for a couple of days, to save his urine for a couple of weeks, have a 1,000 ml. phlebotomy, or just have a few blood samples taken. Somehow, we are all caught in an anthropocentric predicament. Whether we are biologists or just doctors, we intuitively feel that the ultimate subject is man. And there are many respects in which we know we cannot ap-

proach man except by a direct study of man himself.

II. Body size, cooperation, and motivation

The performance of studies of blood often requires samples ranging from 20 to 200 ml. Multiple samples, even of the larger volumes, may be taken from man with relatively little change in his homeostatic adjustment and virtually no harm to him. These are impossible in small animals. This point needs no further elaboration.

Man will cooperate and do what you say. Not always, maybe, but usually. He will run on a treadmill and then stop, hold his breath, lie on his back, stand up until he faints, get on a tilt table, go up to high altitudes or down to deep depths. All of these are merely examples of physiologic experiments that man will do voluntarily. Where they involve actual self-discipline, such as overbreathing, Valsalva's maneuver, starving, thirsting, overdrinking, drug taking, they of course are things that animals cannot do voluntarily.

This voluntary cooperation has another aspect: the question of motivation. A very good example is to be found in the study of muscular strength. Such studies have been done relative to starvation, posttraumatic states, convalescence, rehabilitation, and neuromuscular physiology. The extent to which the individual will exercise his muscle groups depends almost entirely upon the intensity of his motivation. This is very difficult to judge, measure, or control—and may lead to scientific error if improperly evaluated. The same thing is true of studies of pain perception, where the subject himself turns off the stimulant. In evaluating the result of such studies in man, these things must be approached as quantitatively as possible and variables in motivation considered, even in the volunteer.

III. Ethics, Morality, and the golden rule

As we approach the study of man, in our anthropocentric predicament, we must ourselves exhibit the highest qualities of man. These qualities are exemplified, in almost all religious and ethical systems, under the general heading of the golden rule: ". . . whatsoever ye would that men should do to you, do ye even so to them: for this is the law and the prophets." (Matthew 7:12.) There can be no substitute, or any departure from such an ethical approach, in the study of man, as indeed there can be no departure from this rule in the care of the sick.

The horrible nightmares of Dachau and Belsen will ever stand in the conscience of all men, and most especially in the conscience of Germans, as showing what happens when one departs from such a rule. A whole nation becomes prostituted, and science is a mockery. The tragedy of this intentional suffering and torture can never be erased, but one of the ironic tragedies of the human experiments by the German "scientists" was that no good science of any sort came from any of this work.

One cannot help but reflect on the unity of science, art, and religion. Almost all scientists have an artistic appreciation of the beautifully designed experiment and the esthetic satisfaction of the clear concept, the carefully drawn hypothesis, and the well-sculptured conclusion. In their devotion to their work, there is a sense of religious dedication; the basic human traits that go into the making of religion are to be found in science. When a screaming prisoner is plunged into ice water without anesthesia, to see how long before he dies, there is no religion, no art, and no science. It is unfair to beasts to state that in doing such a thing man has reverted to a "bestial state"; no beasts would do such things to other members of their own species save in a struggle for mating predominance. So, although we may try to cross off this terrible chapter in German history, we must constantly be amazed that it has happened in our own time, that it was perpetrated by a nation long productive in science and art, that it is not just a story or myth from tor-

ture chambers of the Middle Ages and that, in this experience, we saw the ultimate prostitution of science. As the Christian ethic was violated, so, too, was science.

As a normal young man enters the hospital for his period of voluntary study these ethical thoughts are in our minds, and we must ask ourselves if the study we are doing exacts any physical, mental, or emotional toll from the volunteer. This problem is approached realistically by categorizing the study as falling into one of three groups:

Group I. Observation only. There may be dietary arrangements and special urinary or fecal collections, but no drugs or injections are given and the patient is merely studied or observed. This involves the least commitment by doctor and subject.

Group II. Injections. When anything is injected into the patient, the study immediately falls into a second category. The hazard of viral hepatitis, of incompatible transfusion reactions, of lethal gram-negative bacteremia, of fatal allergy, anaphylaxis, and sensitization to future therapy must all be considered. Any injection into a volunteer subject must be taken with the utmost seriousness and be fully understood by the recipient. The use of radioisotopes is merely a special category of injection or ingestion, with its own special hazards.

Group III. Loss of voluntary control. This is the third and final step. When a voluntary subject is conscious and in full possession of his faculties, he can always say "No . . . stop." When he is under the influence of sedatives, analgesics, hypnotics, tranquilizers, general anesthetics, or even extreme fatigue, he loses his ability to control what is being done to him. The ultimate moral responsibility is always on the shoulder of the investigator, not the subject; but here, where the subject has lost his voluntary view of the matter, then the investigator cannot help but feel especially strongly the impact of this unshared responsibility.

In our own experience, we have carried out studies in all three categories mentioned above. We approach them with this gradation in mind and with a stepwise precaution, and a very careful explanation to the subject.

IV. Medicolegal

Society attempts to control the effective morality of its citizens through laws. As applied to the study of normal man, these laws are very ill defined, but are operative through the medicolegal codes. Anything that is done to an individual that is not in his self-interest and with his immediate consent constitutes an offense. This legal structure is essential to the practice of medicine, even though it is abused constantly throughout the country by self-seeking people who would rather line their own pockets than seek the truth.

In approaching the study of normal man, medicolegal precautions must be taken. In our experience these have taken three forms:

1. A very careful physical examination and medical checkup of the volunteer subject. We have felt that this was one service we could perform that was helpful to our volunteers and we have always given free of any charge, a complete [illegible] workup. Although there is not space here to define "normal," such a checkup will eliminate obvious abnormality.

2. A signed and witnessed *permission slip* that is completely realistic in all its details. It is not enough for the volunteer to say that he is willing to undergo study. He must specifically say that he is willing to undergo a period of seven days of semi-starvation, with four injections, sixteen blood samples . . . or whatever the details must be. We have also taken special pains to tell our subjects, often in rather discouraging terms, the actual details of our studies so that the reality would be a pleasant rather than an unpleasant surprise.

3. Careful attention to *minors and families.* Carrying out any sort of a medical observation on a volunteer subject under the age of 21 carries special responsibilities and we have never done this without writ-

ten consent from both parents. Furthermore, many individuals over 21 are viewed in a very protective way by their parents or siblings and they are very apt, especially if impetuous college or graduate students, to enjoy doing things on a "dare" without the knowledge of their family. This is a setting for medicolegal disaster. It is therefore essential to be sure that at least one member of the family of any young volunteer knows what is going on.

Although these medicolegal precautions might be regarded as unimportant details when contrasted with the moral and ethical issues at stake, nonetheless they are of vast importance to the individual investigator and his institution. They help to avoid threatening charges which would cast into disrepute the whole field of medical science.

V. Antivivisectionism

Antivivisectionism is a sort of pseudoscientific attack on science, used as a weapon by members of our society who are antirationalistic in many other regards and often emotionally upset. Outright cruelty has no place; but lethal, terminal, or drastic studies may be carried out in anesthetized animals that have no counterpart in the range of clinical investigation. The study of the normal human subject has two very interesting corollaries relative to antivivisectionism.

First, it is very difficult to discuss species differences in medical research without providing arguments that can be taken up by the antivivisectionists. One must be guarded in his statements. The statement that "work in the dog cannot be transferred into man" is apt to be picked up as ammunition by the antivivisectionists, distorted, and used to obstruct all research. Nonetheless, these are part of the hazards of the course. If we cannot face such issues honestly, we had best seek our *veritas* elsewhere. I believe that species differences between dogs and man can be discussed publicly and faced with an honest approach and with sophistication. If this is going to undercut our legislative approach to vivisection, then we should put the latter on a stronger footing. There are so many outstanding instances wherein experiments in the dog have advanced human welfare that this question of species difference need raise no serious issue.

The other possible cross-fire in this area has to do with the fact that the zeal of the antivivisectionists may well find an inviting target in the study of the human volunteer. The latter may be violently opposed on the grounds of inhuman conduct. It is for this reason that the medical, personal, moral, ethical, and medicolegal aspects must be viewed with the greatest respect. Nothing would be more hampering to our institutions than restrictive legislation in this area that is so important and yet so difficult to define.

VI. Reward

Finally we came to the question of "What's in it for the volunteer subject?" Here we have drawn two strict lines.

1. Where the subject is himself scientifically competent to judge the nature of the study, to participate in it as a scientist, and to be a co-author of its publication, there should be no financial payment. If an individual wishes to accomplish an experiment—to ask a question of nature—and wishes to ask this question of his own body, then that is his own problem. He should not be paid for such an act. He is most definitely encouraged to do the experiment, or to have it done by others, and to follow the careful lines of discrimination already outlined. But we regard it as morally wrong then to turn around and pay this individual for having the work done. This seems to be a perversion of scientific curiosity.

2. When the subject derives no scientific, personal, intellectual, or biologic information from the study, then he should be given some reward. Some volunteers do not wish to have any money paid them and that certainly is reasonable enough. If they wish to do it entirely on a voluntary basis, their reward comes from the knowledge of

their help to humanity by the advance of knowledge. Or they may be rewarded by specific personal mention in publications. In many instances volunteers are students or young people with financial problems, and in many of our studies they have carried on work for their thesis while in the hospital, feeling that they could earn some "pin money" this way. We have therefore often paid our volunteers with the hope that this money would help them with their education. The amount that we have paid them has been variable and has been determined by the length of time they were inconvenienced or restricted in activity because of the study and the actual personal discomfort involved in such things as blood samplings or dietary restrictions. In general we have tried to make these payments large enough so that they did make some contribution to the welfare of the individual, but we have tried to avoid getting on an inflationary escalator by which the payments became progressively larger and out of proportion to the meager funds available for research. In individuals who are going to pursue a medical career with its high ambient radiation flux, we have avoided the use of radioactive isotopes regardless of other considerations.

VII. Summary

No single summary is possible. We may bring together some of our points relative to scientific study of human volunteers, as follows:

1. Species differences among vertebrates are especially noticeable in body composition, whole-organ function, and integrative systems. These make the study of man essential in biomedical research.

2. Body size, motivation, and cooperation make man a desirable subject.

3. The highest ethical and moral standards must be employed in studying normal human volunteers. Observation alone, injection, and loss of voluntary control represent three contrasting and progressively more serious aspects of responsibility for the investigator.

4. Medicolegal precautions must be taken.

5. Antivivisectionism must not be served by incautious pronouncement.

6. Reward must be considered realistically.

(To be continued.)

Nitrofurantoin (Furadantin) in the biliary tract

Twiss and his co-workers reported that the oral administration of nitrofurantoin produced bactericidal levels of the drug in the bile. The organisms which infect the biliary tract are frequently the same as those which infect the urinary tract. They therefore inferred that nitrofurantoin should be a valuable agent in treating infections of the biliary tract. We were unable to eradicate infections in the biliary tract even though the organisms isolated from culture of the bile were sensitive to the action of nitrofurantoin in vitro. Therefore we decided to repeat the studies of Twiss and his associates.

Following the oral administration of adequate doses of nitrofurantoin, the levels of this drug were studied in bile specimens obtained from the gall bladder by T-tube drainage and duodenal tube drainage. Blood levels were also determined. Nitrofurantoin levels were far below those obtained by Twiss. In no instance was there any bactericidal activity of nitrofurantoin observed at levels present in the bile during treatment. Nitrofurantoin is therefore ineffective in treatment of infections of the biliary tract.

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Twiss and co-workers[1] administered 100 mg. of nitrofurantoin* orally to patients 4 times per day and reported finding bactericidal levels of the drug in bile specimens obtained at the time of operation either by T-tube drainage of the common duct post cholecystectomy or by duodenal tube drainage. The inference was drawn that nitrofurantoin was bactericidal in infections of the biliary tract. In fact, these workers had started to use nitrofurantoin therapeutically in chronic cholangitis, but their results have not yet been reported. Since in our experience we found no clinical or bacteriologic improvement in a number of patients with cholangitis following nitrofurantoin treatment, although the pathogen cultured from the bile was extremely sensitive in vitro to nitrofurantoin as tested by the standard paper-disc method, we decided to repeat the studies of Twiss and his co-workers and at the same time to test the clinical effectiveness of this drug as a bactericidal agent in the biliary tract.

Nitrofurantoin is a nitrofuran, N-(5-nitro-2-furfurylidene-1-aminohydantoin). It is excreted in high concentration in the urine. It has proved to be one of the most effec-

*Furadantin.

tive bactericidal agents in urinary tract infections where the pathogen is often similar to that found in the biliary tract.

Method of study

Twenty-five patients were selected for this study.

Gall bladder bile. Fourteen patients admitted to the hospital for cholecystectomy were studied. All had cholelithiasis, cholecystitis, or both. Some were ill with cholangitis. One or 2 days preoperatively, 100 mg. (occasionally 200 mg.) of nitrofurantoin was given orally after meals and at bedtime. At the time of cholecystectomy a specimen of venous blood and of bile was obtained, the latter from the gall bladder by needle aspiration. The bile was cultured. If a pathogen was present its sensitivity was tested by the standard paper-disc method to the following antibacterial agents: penicillin, chloramphenicol, polymyxin B, oxytetracycline, erythromycin, tetracycline, dihydrostreptomycin, chlortetracycline, kanamycin, novobiocin, and nitrofurantoin. The nitrofurantoin concentrations in the specimens of bile and blood were determined and the results are shown in Table I.

Liver bile. Nine patients were studied, all with T-tube drains in the common duct post cholecystectomy. For 1 to 7 days preceding the test, 100 mg. of nitrofurantoin was given orally after meals and at bedtime. On the morning of the test, 12 hours after the last dose of nitrofurantoin, a specimen of venous blood and a specimen of bile were obtained, the latter from the T-tube. Another 100 mg. of nitrofurantoin was given. Specimens of blood and bile were obtained 2, 4, and 6 hours later. Whenever possible the bile specimens obtained before and after the administration of nitrofurantoin were cultured. The sensitivity of the pathogens to various antibacterial agents was studied by

Table I. *Specimens of blood and gall bladder bile obtained by aspiration at cholecystectomy*

No.	*Patient*	*Concentration of nitrofurantoin (μg/ml.)*		*Culture of bile*	*Sensitivity of organism*
		Bile	*Blood*		
1	M. P.	7.80	6.25	Sterile	—
2	C. D.	1.56	10.37	Sterile	—
3	E. P.	1.95	6.25	Sterile	—
4	B. M.	1.95	2.58	Sterile	—
5	W. R.	6.25	10.37	*Escherichia coli* *Aerobacter aerogenes*	Chloramphenicol Streptomycin Polymyxin B Nitrofurantoin
6	S. W.	1.95	6.25	Sterile	—
7	V. C.	<1.56	7.81	Sterile	—
8	H. S.	1.56	10.37	*Salmonella typhi*	Chloramphenicol Polymyxin B Tetracycline Oxytetracycline Chlortetracycline Nitrofurantoin
9	R. Z.	<1.56	7.81	Sterile	—
10	L. L.	1.56	8.87	*A. aerogenes*	Chloramphenicol Kanamycin Streptomycin Nitrofurantoin
11	C. R.	6.25	12.81	Sterile	—
12	R. P.	6.25	5.19	Sterile	—
13	C. L.	7.81	10.37	Sterile	—
14	A. S.	1.56	7.81	Sterile	—

Table II. *Specimens of blood and common duct bile obtained by T-tube after cholecystectomy*

No.	Patient	Culture of bile	Sensitivity of organism	Concentration of nitrofurantoin (μg/ml.)							
				Bile				Blood			
				Hours after fourth dose	Hours after fifth dose			Hours after fourth dose	Hours after fifth dose		
				12	2	4	6	12	2	4	6
15	S. W.	*Aerobacter aerogenes*, beta hemolytic streptococcus, *Bacillus pyocyaneus*	Polymyxin B Streptomycin Nitrofurantoin	1.56	1.56	1.56	1.56	2.58	2.58	2.58	2.58
16	S. G.	Sterile	—	2.58	1.56	1.95	—	5.19	2.58	2.58	—
17	L. N.	*A. aerogenes*	Chloramphenicol Streptomycin Nitrofurantoin	—	1.56	1.56	—	—	2.58	2.58	—
18	A. I.	*A. aerogenes*	Polymyxin B Kanamycin	7.81	7.81	7.81	6.25	—	15.62	9.9	9.9
19	B. N.	*Alkaligenes faecalis*	Chlortetracycline Tetracycline Oxytetracycline Kanamycin	—	1.56	1.56	—	—	10.37	5.19	—
20	S. S.	Contaminated	—	—	1.56	1.56	—	—	7.81	2.58	—
21	K. M.	Contaminated	—	1.56	1.56	1.56	—	2.58	7.81	5.19	—
22	H. T.	Sterile	—	1.56	1.56	—	—	1.56	7.81	—	—
23	F. M.	Contaminated	—	—	5.19	—	—	—	12.90	—	—

the standard method. The nitrofurantoin concentrations in the specimens of bile and blood were determined and the results are shown in Table II.

Duodenal bile. Duodenal intubation with a Jutti tube was carried out in 2 patients with the procedure described by Twiss and Oppenheim.[2] The bile was cultured and the bacterial sensitivity studied by the standard method. Then 100 mg. nitrofurantoin was administered 4 times daily for 2 days, and duodenal drainage repeated 12 hours after the last dose. Bile and blood specimens were obtained, and 200 mg. of nitrofurantoin was again given. Specimens of bile and blood were obtained 2, 4, and 6 hours later. Whenever possible the bile specimens were cultured and the sensitivity of the pathogens determined. The nitrofurantoin concentrations in the specimens of bile and blood were determined. The results are shown in Table III.

Two cases will be reported. S. W. (Case 15) received long-term therapy with nitrofurantoin both orally and intravenously. Numerous bacteriologic cultures of the bile were made and nitrofurantoin levels in blood and bile were determined at frequent intervals before and after operation. The results are shown in Table IV. S. B. W., a sec-

ond patient, also received long-term therapy but was studied only clinically and by cultures of bile obtained by duodenal tube drainage. This patient is not included in the tabulated cases.

Determination of nitrofurantoin activity

The concentration of nitrofurantoin in bile and blood, and the antibacterial activity of nitrofurantoin in the bile were determined by the test tube dilution method as originally outlined for penicillin concentration in body fluids by Rammelkamp.[3]

Preparation of the standard. One hundred milligrams of nitrofurantoin was dissolved in 10 c.c. of N,N-dimethyl-formamide, the resulting solution was diluted with 99 volumes of McIlvaine's buffer, and then twofold serial dilutions in tryptose-phosphate broth were made to obtain a solution of nitrofurantoin ranging from 0.39 to 100 mcg. per milliliter. All standards were sterilized by Seitz filtration.

Test organism. Several strains of *Escherichia coli* were tested for sensitivity to the above range of nitrofurantoin concentrations and the most sensitive strain was chosen as the test organism. The smallest concentration of nitrofurantoin which prevented growth of this organism was 1.56 mcg. per milliliter.

Preparation of the sample. Sterilization of the bile specimen was necessary when testing for nitrofurantoin concentration to prevent extraneous bacteria from interfering with the test organism. This could not be accomplished by autoclaving since it was found that by this method no appreciable nitrofurantoin activity could be obtained. (We subsequently learned that many organic substances, such as body fluids and other active organic materials such as McIlvaine's buffer and tryptose-phosphate broth which we used as diluents, may decompose nitrofurantoin when they are heated together. However, nitrofurantoin is not altered by heat when combined with water or inert organic substances such as Carbowax). The Seitz filtration method of sterilization was therefore used. Study of filtered and unfiltered control specimens revealed that this process did not interfere with nitrofurantoin activity. Corresponding blood specimens were allowed to clot and the sera were drawn off with sterile precautions. In anticipation of the possibility of having to keep specimens in the refrigerator

Table III. *Specimens of blood and bile obtained by duodenal-tube drainage*

No.	*Patient*	*Culture of bile*	*Sensitivity of organism*	*Concentration of nitrofurantoin (μg/ml.)*							
				Bile				*Blood*			
				Hours after fourth dose	*Hours after fifth dose*			*Hours after fourth dose*	*Hours after fifth dose*		
				12	2	4	6	12	2	4	6
24	S. P.	Beta hemolytic streptococcus *Aerobacter aerogenes*	All broad spectrum antibiotics	1.56	1.56	<1.56	<1.56	1.56	2.58	2.58	2.58
25	T. R.	*A. aerogenes*, gamma streptococcus	All broad spectrum antibiotics, nitrofurantoin	1.56	1 56	5.19	—	—	1.95	2.58	—

Table IV. *Specimens of blood and bile in patient S. W. (Case 15)*

Date	*Days of treatment*	*Source of bile*	*Culture of bile*	*Sensitivity of organism*	*Maximum Concentration of nitrofurantoin (μg/ml.)*	
					Bile	*Blood*
6–30–58	0	Cholecystostomy	*A. aerogenes*	Nitrofurantoin Kanamycin Polymyxin B	0	0
7– 2–58	2	Cholecystostomy	*A. aerogenes*	Kanamycin Nitrofurantoin Polymyxin B	1.56	—
7– 7–58	6	T-tube	*A. aerogenes*	Nitrofurantoin Kanamycin Polymyxin B	1.56	—
7– 8–58	7	T-tube	*A. aerogenes* Beta hemolytic streptococcus	Nitrofurantoin Kanamycin Polymyxin B Streptomycin	1.95	—
7–10–58	9	T-tube	*A. aerogenes* Beta hemolytic streptococcus *B. pyocyaneus*	Nitrofurantoin Kanamycin Polymyxin B Novobiocin Oxytetracycline	1.56	2.58

before processing them, standard amounts of nitrofurantoin were prepared and divided into 2 aliquots. One was run the same day, others were kept in the refrigerator at 4°C. for up to 5 days before testing. There was no appreciable loss of nitrofurantoin activity even after 5 days.

Each set of bile and blood specimens was run simultaneously. Using the standard tube-dilution procedure, each specimen was dispersed into sterile test tubes in decreasing amounts of 1 ml., 0.9 ml., 0.6 ml., 0.3 ml., and 0.1 ml. All tubes containing less than 1 ml. were filled to that volume with tryptose-phosphate broth. One drop of a 24 hour culture of the test organism, diluted 1 to 100, was added to each tube. The tubes were incubated at 37° C. for 24 hours. The lowest dilution showing no growth was taken as the end point. The nitrofurantoin concentration was determined from the sample by comparison with the sensitivity of the test organism. Thus, if the tube containing 1 ml. of the unknown is the lowest dilution inhibiting growth of the test organism (the end point), it must contain 1.56 mcg. of nitrofurantoin per milliliter (sensitivity of the test organism). If the tube containing 0.5 ml. of the unknown is the end point, it will contain 3.125 mcg. per milliliter. If the tube containing 0.1 ml. is the end point, it will contain 15.6 mcg. per milliliter.

Illustrative case reports

Case 1. S. W., a 38-year-old man, was operated upon at Beth-El Hospital May 8, 1958. Cholecystostomy was performed for gangrenous cholecystitis and acute hemorrhagic pancreatitis. Convalescence was uneventful. He was discharged May 24. He was readmitted June 30. Culture of the bile from the cholecystostomy tube showed a pure growth of *Aerobacter aerogenes,* very sensitive to nitrofurantoin and tetracycline, as tested by the standard paper-disc method.

He was treated with 100 mg. of nitrofurantoin 4 times daily until July 2 when a cholecystectomy was performed and a T-tube inserted in the common duct. For the first 4 postoperative days he was given 480 mg. of nitrofurantoin intravenously daily. Beginning July 6, peroral treatment was resumed with 100 mg. of nitrofurantoin 4 times daily. He was discharged on July 12 with the T-tube in situ. On July 3, 7, 8, 10, and 12 bile was cultured and the nitrofurantoin level determined. *A. aerogenes* was always cultured from the bile and the nitrofurantoin levels in the blood and bile were always low (Table IV). After discharge the patient continued to take 400 mg. of nitrofurantoin daily. On July 26 he developed pain in the right upper quadrant, chills, and an elevated temperature. Nitrofurantoin was discontinued and 250 mg. of tetracycline was given every 6 hours. The symptoms subsided within 24 hours. Bile culture done one week later was sterile. Antibiotic therapy was continued until September 1 when the T-tube was removed from the common duct. The drainage wound closed quickly and therapy was discontinued. Convalesence thereafter was uneventful.

Case 2. S. B. W., a 55-year-old man, was admitted to the New York Polyclinic Hospital on May 3, 1958, for acute cholangitis [illegible] obstructive jaundice caused by a calculus in the common bile duct. Bile obtained by duodenal drainage contained many polymorphonuclear leukocytes and cholesterol crystals. *A. aerogenes* and *E. coli* were cultured from the bile. Penicillin and streptomycin were given parenterally, despite which the spiking temperature and jaundice persisted for 4 days. On May 6 the temperature fell to normal and the jaundice began to subside. On May 8 bile drainage still showed many polymorphonuclear cells and the organisms previously found were again cultured from the bile. Nitrofurantoin, 100 mg. 4 times daily, was administered orally for a week. Jaundice and fever subsided and the patient felt well. However, bile obtained by duodenal drainage on May 16 still contained many polymorphonuclear cells and on culture revealed the same organisms. Operation was advised but refused. The patient was discharged but continued to take nitrofurantoin for another 10 days. Bile drainage on May 26 showed no change in the microscopic or bacteriologic findings. It is obvious that in this case neither the antibiotic nor nitrofurantoin has been able to clear the persistent infection. Symptomatic relief has been temporarily obtained because the obstruction corrected itself spontaneously.

Discussion

It might be assumed that nitrofurantoin, which is so effective as a bactericidal agent in infections of the urinary tract, would also be of value in the treatment of infections in the biliary tract because in many instances the same species of pathogenic organisms are involved. It is known, however, that nitrofurantoin activity varies with the pH. It is very active in an acid medium and inactive in an alkaline medium. The pH of the urine is usually acid, that of the bile always alkaline. This fact alone would militate against its usefulness in biliary tract infections. It might be argued that if large enough concentrations of nitrofurantoin could be excreted in the bile, then perhaps the quantitative effect could somehow overcome the adverse effect of an alkaline medium. The results presented in Tables I-IV show minimal excretion of nitrofurantoin in the bile. At times no biliary excretion of the drug was found. The nitrofurantoin levels in the bile and blood were less than 5 per cent of those noted by Twiss and associates.[1] In no instance have we been able to eradicate infection in the biliary tract, although in many cases the infecting organism was highly sensitive to nitrofurantoin when tested by the standard paper-disc method. In the first case reported, nitrofurantoin was ineffective both clinically and bacteriologically. In fact, acute cholangitis developed while the patient was taking adequate amounts of nitrofurantoin over an extended period of time. In the second case, subsidence of clinical symptoms apparently

paralleled the relief of the common duct obstruction since nitrofurantoin, penicillin, and streptomycin all failed to eliminate the infection. In 3 other cases of cholangitis treated with nitrofurantoin, but in which no laboratory studies were performed, no clinical improvement was noted. It can therefore be concluded that nitrofurantoin is ineffective in the treatment of infections of the biliary tract.

Summary

1. The excretion of nitrofurantoin in the bile and its clinical effectiveness as a biliary bactericidal agent were studied by detailed laboratory examination in 25 patients. Gall bladder bile was obtained at the time of cholecystectomy in 14 patients, from T-tube drains in the common duct in 9 patients and by duodenal intubation in 2. Blood samples were obtained simultaneously with the bile specimens and nitrofurantoin levels in the bile and blood were determined. Bacteriologic studies of the bile were made before and after the administration of nitrofurantoin. The case history of one patient, who received prolonged treatment, and in whom frequent laboratory examinations were performed, is presented in detail.

2. In no instance was there any bactericidal activity of nitrofurantoin observed at levels present in the bile during treatment and the drug was ineffective in the treatment of infections of the biliary tract.

3. Nitrofurantoin levels in bile and blood were far below those obtained by Twiss and his co-workers.

4. There was no favorable clinical response in 3 additional patients with acute cholangitis, treated over long periods of time with nitrofurantoin. In another case there appeared a clinical response without bacteriologic improvement, the response being probably coincidental and due to spontaneous removal of obstruction.

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Clinical pharmacology of analgesics

1. A method of assaying analgesic effect

Animal screening tests and methods employing induced pain in human volunteers have failed to predict with any consistency the clinical performance of analgesic drugs. These drugs must be assayed in a clinical setting in which they might be used therapeutically. The lack of adequate objective standards for the measurement of pain or pain relief, the variability in responses among patients, and the influence of environmental factors on these responses, all make it essential that the study be adequately controlled and so designed as to provide some measure of the sensitivity of the method and of the statistical significance of the results.

Such a method for evaluating analgesics in patients with chronic pain due to cancer has been employed at the Memorial Cancer Center for the past 8 years. Measurements of analgesia are derived from the patients' own estimates of pain intensity both before and at hourly intervals after a test medication yielding data which allow comparison of drugs in terms of peak and total effects. Using these criteria, studies of the effects of morphine [illegible] a placebo were carried out and provide the basis for a method of assaying other analgesic [illegible]rugs. A prototype of the latter is presented in the form of a study of aspirin, morphine, [illegible]d a combination of these drugs.

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The clinical evaluation of any potential analgesic begins with the determination of whether or not the drug has an analgesic effect in man. It must be shown that this effect is due to the drug rather than to extraneous factors which may influence pain.

Testing of analgesics is fundamentally different from that of most other classes of drugs. One can determine whether or not a drug is an antibiotic or a vasopressor in the laboratory regardless of whether or not it is effective in the treatment of human infections or shock in medical practice. On the other hand, there are no completely reliable screening tests for analgesics in the laboratory and one must therefore turn to

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the clinical situation. Beecher[1] has discussed this in detail in a comprehensive review on testing of analgesics. The therapeutic trial constitutes an essential preliminary step in determining the clinical pharmacology of this class of drugs (the local and general anesthetics are not generally included in this group), for the mere classification of a drug as an analgesic rests solely with the demonstration of its ability to relieve pain in the clinical situation.

Yet a great deal of confusion about the clinical effectiveness of many analgesic drugs is evident in the medical literature. It is the rule rather than the exception to find that early clinical reports have tended to exaggerate the effectiveness of new analgesics in the light of subsequent experience. Unfortunately there have been few adequately controlled studies using sufficiently well-defined criteria to permit objective evaluation of results and conflicting statements by different investigators cannot be easily resolved.

This report is based on 8 years of experience with a method of assaying analgesics in patients with chronic pain at the Memorial Center for Cancer and Allied Diseases.[3,9] Our primary concern was to develop a method which would give us objective, reproducible, and precise information on analgesia in the clinical setting.

It is impossible to approach this problem without making certain basic assumptions.

An estimate of analgesic effectiveness requires some measure of the presence or absence (and preferably an estimate of the severity) of pain in the patients under study, both before and after receiving analgesic drugs. There is no universally accepted yardstick by which to measure the phenomenon. Pain is primarily a subjective experience with variable and unreliable objective manifestations. The best estimates of pain intensity are, we feel, the reports of the patients themselves. Reliable information can be obtained from subjective data of this sort when proper controls and checks are employed. We have felt it essential, therefore, that these studies be carried out in accordance with the principles of double blind and controlled experimental design.[4]

Certain drugs are universally accepted as analgesics. In the absence of satisfactory objective criteria, we have merely accepted this as so, and used as a test of sensitivity of our method the ability of our assay to distinguish between these drugs and pharmacologically inert materials in terms of analgesic effect.

Methods

Design. The subjective nature of these data places several restrictions on the study reflected in the design of the experiment. Each patient must evaluate his pain experience in his own terms of reference. The cross-over design has been employed as the most efficient way of matching for age, sex, and psychological factors the relatively small numbers of patients drawn from our limited hospital population. Each patient in our study serves as his own control. All medications used are treated as "unknowns" and a placebo included to determine the base line of positive suggestion therapy. The drugs are prepared in such a form as to appear indistinguishable one from another and are identified only by code letters. To avoid even unconscious influence on the patient's responses, the observer as well as the patient is kept in the dark as to the identity of the medications (double blind technique).

Selection of patients. Subjects are selected for our studies from among the population of patients with advanced cancer who are on the wards of the James Ewing Hospital. Well-oriented, cooperative patients who report pain due to their disease and for whom there are no medical contraindications to the type of drugs that we are testing are asked to volunteer for the studies. Physical examinations of all patients are carried out and only those whose reports of pain appear to be consistent with evident or demonstrable disease are selected. The patients are informed that they will be given a variety of drugs, including some new ones,

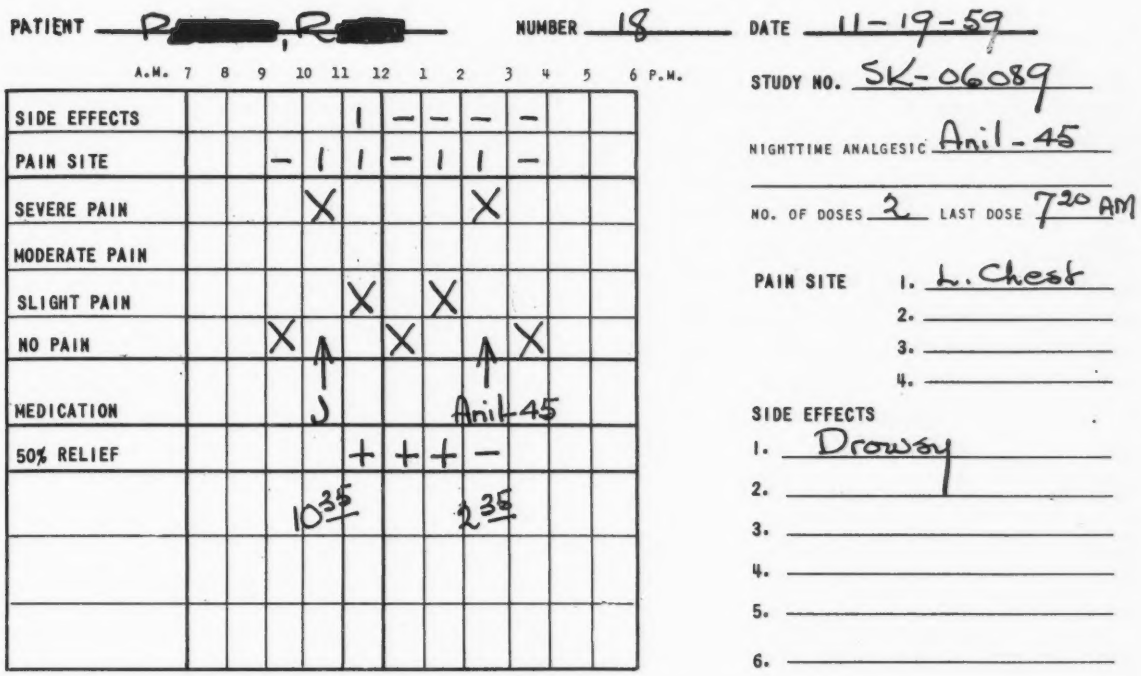

Fig. 1. Daily pain chart. An example of the basic information obtained by the nurse-observer in the course of her hourly interviews of each patient-subject each day. The patient's report of the site and severity of his pain, the administration of any coded test drug or regular medication, whether or not he believes his pain to be at least half relieved by medication, and any evident or volunteered side effects are noted in the appropriate time columns at each interview.

and the scale by which they are to rate their pain is explained to them.

Collection of data. A registered nurse (A. R.) serves as full-time observer and interviews the patient-subjects on the wards at hourly intervals during the daytime observation period (10 hours a day, 5 days a week). Patients are questioned to determine the presence and severity or absence of pain. The questioning is informal, but leading questions are studiously avoided. When the patients require medication for pain, coded test medications are administered according to a randomized dosage schedule. No test drug is administered within 3 hours of a previous analgesic medication. Observations are made for an arbitrary 6 hour period after administration of the test drug, or until pain returns to the premedication level and an additional analgesic is required.

The observations are recorded on a daily pain chart (Fig. 1) similar to that developed by Keele.[5] The categories of slight, moderate, or severe pain all represent subjective judgments of the patients themselves. We recognize that any categorization of such a subjective reaction as the continuum of pain intensity will inevitably be gross and have ill-defined parameters. Indeed, these categories are not discrete ones and may well have different meanings to different patients. It was hoped that each patient would be reasonably consistent within himself during the relatively short period of time needed to study three or four test drugs. In addition to the hourly reports of pain intensity, provision is made on the pain chart for reporting the time of administration and code letter of the test drug, any apparent or volunteered side effects, and information on the site of pain. The times of administration of all analgesics given before or after the test period are also

recorded. Patients are also asked to report whether or not the medication relieved at least 50 per cent of their pain, so that the quantitative data in terms of pain severity may be compared directly with the type of qualitative data obtained by other investigators.[1]

As we do not accept sleep as indication that the medication may have produced an analgesic effect, patients are awakened if necessary at each observation period. We have not found it practical to extend our observations into the usual sleeping hours for this reason and also because the use of more than one observer would require a more complex experimental design or some duplication of observations to provide an estimate of observer-patient variables. Only a single observer was used in these studies.

Handling of data. The data are analyzed by persons not actively participating in making the observations. Information on pain intensity for 6 hourly intervals after medication is converted into hourly pain relief scores. In each hour, one point of pain relief is scored for each category drop in pain below the premedication pain level.

In Fig. 1, for example, an actual record of patient R. P., the coded drug "J" was administered when the patient complained of *severe pain.* One hour later the pain had dropped two categories to *slight pain* for a score of 2 points. In the next hour there was a further drop to *no pain* for a score of 3 points in this hour. In the third hour after drug, *slight pain* had returned so that the score was 2 points for that hour also. Four hours after drug the pain had returned to the premedication level and a known analgesic which was not being evaluated was administered. No relief points were scored for drug "J" for this or for subsequent hours.

We would prefer not to have to give other analgesics for 6 hours after the test medication but this is hard to justify in dealing with sick patients in pain. We do, however, try to withhold other medication for at least 2 hours and until the patient's pain returns to the premedication level. An increase in pain after medication (from *moderate* to *severe pain*) is scored simply as no relief.

The hourly relief scores thus obtained yield quantitative information on peak drug action, duration, and the time effect curves of the drugs. By totaling the 6 hourly relief scores for each drug, an estimate is obtained of the area under the curve in the form of *total relief scores.* These latter scores, which may range from 0 (no relief) to 18 points (complete relief for 6 hours) are the units we have usually employed for the statistical analysis of drug effects.

Validation of method. Before the testing of new drugs was undertaken, the validity, reliability, and sensitivity of the method were evaluated in a study comparing an accepted standard analgesic and a placebo. A comparison of the effects of 10 mg. of morphine sulfate and a sterile saline placebo, both administered intramuscularly, was carried out by the method outlined above in 67 patients. Both drugs were administered under double blind conditions in a random order to each patient. Information was obtained on both hourly fluctuations in pain intensity and whether or not the patients considered their pain at least half relieved.

Morphine and aspirin study. A comparison of the analgesic effects of morphine, aspirin, and a combination of morphine and aspirin was undertaken in a study which serves as a prototype of the factorial assays we have carried out to determine the presence or absence of analgesic properties of new drugs. A placebo, 600 mg. of aspirin by mouth, 10 mg. of morphine sulfate intramuscularly, and the combination of 600 mg. of aspirin and 10 mg. of morphine were the test medications. To maintain double blind conditions, both capsules and an injection were included in each drug administration. The placebo consisted of a sterile saline injection and two lactose capsules. Sterile saline was also administered with the aspirin, and lactose with the morphine so that these medications also appeared identical. Twenty-eight patients received at least one dose of each medication. Sixteen of these

patients received each drug a second time (replicated data), for a total of 44 doses of each medication. The collection and handling of the data were carried out as described above.

Results

Comparison of the analgesic effects of morphine and a saline placebo. A summary of the data obtained in our first group of patients is presented in Fig. 2. The data are plotted as *time-effect curves* in terms of two parameters of analgesic effect: (1) the mean hourly pain relief scores derived from the patients' estimates of pain intensity, and (2) the percentage of patients reporting relief of at least half of their pain at each hour after medication. Since these measures of analgesia are not strictly comparable, the vertical ordinates in the figure were drawn in such a way as to show readily the relative effects of morphine and the placebo by both parameters of effect. It is evident that the time-effect curves for the individual medications are very similar by either criterion, so that it may be assumed that as long as the *relative* effects of medications are compared, one should not expect very different results whether one chooses to employ a quantitative measure of analgesic effect (graded scale of pain intensity or pain relief) or a quantal one (whether or not the pain is at least half gone).

It is to be noted that the sterile saline placebo had an appreciable effect and that its time-effect curve mimicked that of morphine. However, the 10 mg. dose of morphine produced considerably more relief than the placebo in these patients for at least 5 hours after administration. In this study, the peak effect was noted at the first hour and these values for morphine and saline were 1.5 and 0.9, respectively, in terms of relief scores, and 63 and 41 per cent, respectively, in terms of per cent of patients reporting at least half relief of their pains. Placebo was therefore about one-third less effective than morphine by either index of effect. As will be discussed below, however, neither the relative nor the actual values obtained for the drugs are especially meaningful as such, for they are not constant from one patient group to another (when dealing with relatively small patient-population samples), and the important statistic is whether or not the difference is significant, i.e., not likely due to chance.

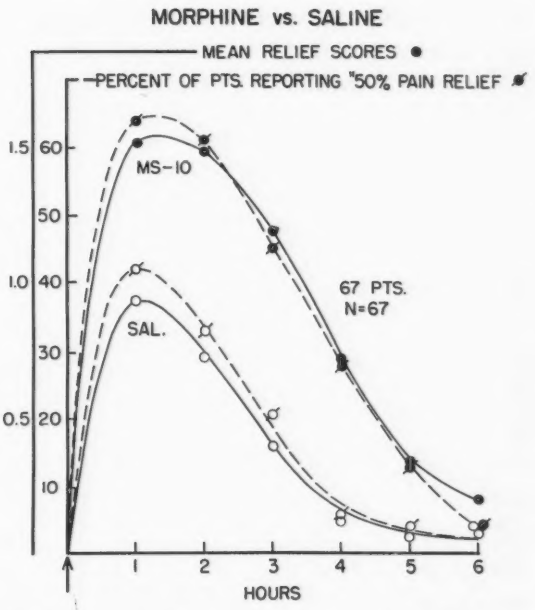

Fig. 2. Time-effect curves for morphine sulfate 10 mg. intramuscularly (dots) and sterile saline intramuscularly (circles) in terms of both the mean pain relief scores (solid lines) and the percentage of patients reporting at least 50 per cent relief of pain (broken lines) at each hour after medication. Each point represents the responses of the same 67 patients.

A convenient way of demonstrating the variations in responses to the placebo and morphine among the patients of this study is shown in Fig. 3. This is a *scatter diagram* of the total relief scores of each patient to both drugs, each point (xy) representing the morphine score (y axis) and the placebo score (x axis) for a single patient. This type of analysis, of course, can be carried out only on quantitative data and the additional information that it gives about the study emphasizes one of several advantages of using the pain scores in preference to the "half-relieved" criterion of measuring effect.

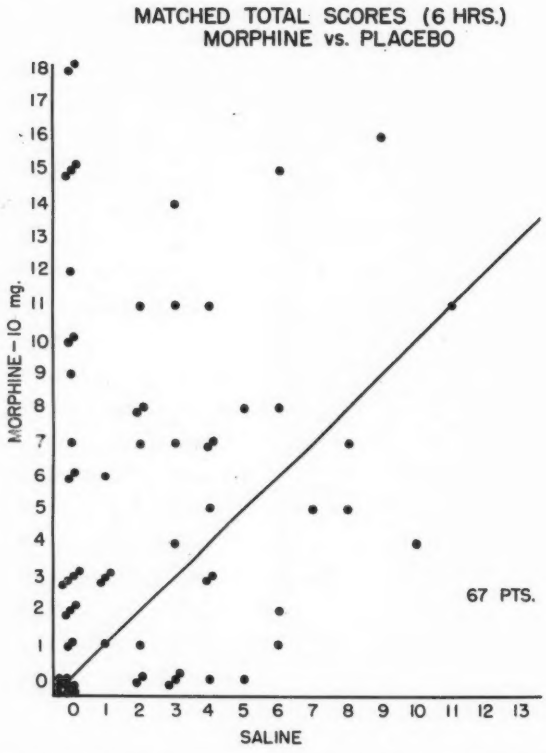

Fig. 3. Scatter diagram of the responses of each of the 67 patients to both the morphine sulfate 10 mg. and the sterile saline injections (intramuscular). Individual points indicate the total relief scores for morphine and the placebo in the same patient. The vertical ordinate represents the morphine scores and the horizontal ordinate the saline placebo scores. The diagonal line separates those who scored higher with morphine than with the placebo (above line) from those who scored higher with the placebo than with morphine (below line).

The scatter diagram demonstrates several things in addition to the obvious fact that these patients differed considerably one from another. Pertinent to the issue of assessing the validity of a method of assaying analgesics is whether or not the patients are capable of discriminating between a known analgesic and a placebo. Of these 67 patients, 41 obtained more relief with morphine (points above the diagonal line), 10 obtained equal scores with morphine and saline, and 16 obtained better scores with saline. In the absence of ability to discriminate there is an equal chance of scoring better with one medication than the other, and we must therefore assume that as many nondiscriminators scored better with morphine as scored better with saline. This leaves 41–16=25, or approximately only 37 per cent of the group whom we can consider to be discriminators.

It is also to be noted that the level of the placebo response was not in itself a measure of the ability of these patients to discriminate. Many patients who reported some relief with saline obtained even greater relief with morphine, and some patients who obtained no relief from the placebo reported no relief from morphine either. If we had been able to screen out placebo reactors from this group, 57 per cent of the patients would have been eliminated. The problem of eliminating "placebo reactors," however, is difficult and impractical for still other reasons: in surveying our experiences over the past 8 years, we have found that over 90 per cent of our patients reported some relief from placebos at some time or another. It would appear that the pertinent issue concerns *placebo reactivity* rather than placebo reactors. Thus any attempt on our part to screen out placebo reactors in advance would have greatly restricted the number of patients available for study and, in the process, would also have eliminated many patients who are good discriminators. This would actually have resulted in decreasing rather than increasing the sensitivity of our method.

Effects of graded doses of morphine. Another measure of the adaptability and sensitivity of the method is the ability to demonstrate graded effects from graded doses of a known effective analgesic. Fig. 4 summarizes the results of such a study in a group of 10 patients who received doses of 5, 10, 15, and 20 mg. of morphine sulfate and a saline placebo. The data show that by varying the doses of the drug several parameters of drug effect are affected: the configuration of the time-effect curves, peak and total effects, and the duration (and very likely the onset) of effect. Though a placebo was included in this study and

its effect was appreciably lower than that of 5 mg. of morphine, the mere demonstration that a significant slope was obtained for graded doses of morphine in terms of both the peak and total effects in itself serves as an internal control of the sensitivity of the method. The fact that the method is this sensitive opens the way for comparative studies of graded doses of drugs and relative potency assays can then be carried out.

Comparison of the analgesic effects of morphine and aspirin. The purpose of this study was to compare the effects of a placebo, 600 mg. of aspirin, 10 mg. of morphine sulfate, and the combined administration of both aspirin and morphine. This represents all possible combinations of the test medications, and is spoken of as a *factorial* experiment. Since we intended to assay the effects of these drugs in the form in which they are conventionally used, each test medication included both an injection and capsules by mouth so as to maintain double blind conditions.

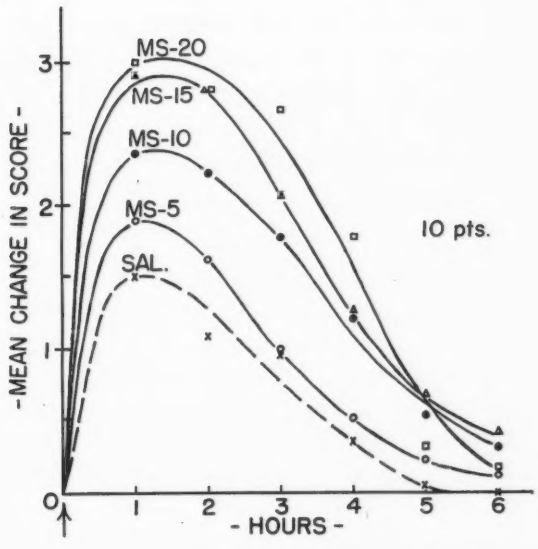

Fig. 4. Time-effect curves for graded doses of morphine sulfate (5, 10, 15, and 20 mg. intramuscularly) and a sterile saline placebo in terms of the mean pain relief scores at each hour after medication. Each point represents the average response of 17 administrations of each medication in 10 patients (7 patients received all medications twice, and 3 received them all once).

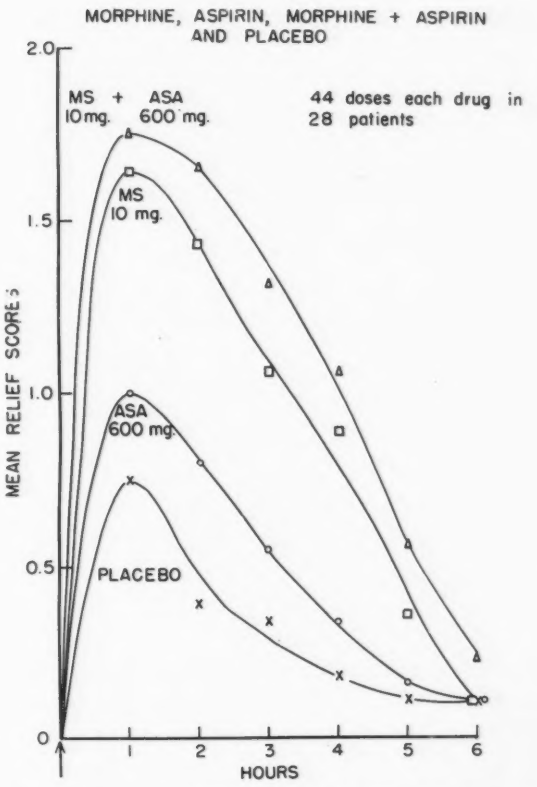

Fig. 5. Time-effect curves for the placebo (lactose by mouth plus sterile saline intramuscularly), 600 mg. aspirin by mouth (plus sterile saline intramuscularly), 10 mg. of morphine sulfate intramuscularly (plus lactose by mouth), and the combination of 600 mg. aspirin by mouth plus 10 mg. morphine intramuscularly, plotted as mean pain relief scores at hourly intervals after drug administration. Each point represents the responses to 44 doses of drug in 28 patients (16 received all medications twice, 12 received all medications once).

The pain relief scores obtained with these medications are plotted as time-effect curves in Fig. 5. As has been evident in all of our studies, the placebo again mimicked the active drugs, but a clear separation of effect was also noted for each of the test medications. Of particular interest in terms of the sensitivity of the method were the observations that aspirin proved superior to the placebo, and that aspirin plus morphine was better than morphine alone.

Table I. *Morphine vs. aspirin. Analysis of variance*

Source of variation	*Degrees of freedom*	*Mean squares*	*F ratio*
Patients	27	27.1	3.47*
Treatments	3	214.6	27.51*
Replications	1	21.1	2.71
P × T	81	8.7	
P × R	15	6.2	
T × R	3	12.0	
P × T × R	45	6.4	
Total	175		
Combined interactions	144	7.8†	

*Significance level: $p < 0.01$.
†Assay error.

For purposes of testing the statistical significance of these results, the total effects of these medications were analyzed. Table I shows the results of the analysis of variance[8] of these data. The major sources of variation are the patients, treatments (medications) and replications (16 patients received all medications twice), and their interactions. Since the variances of the latter were approximately of the same magnitude as the residual or random variance (P × T × R), they were combined and used as the assay error. By this test, the variances among patients and among treatments were both highly significant ($p<0.01$), whereas that between replications was insignificant. The results of this analysis are typical of those obtained in our studies. They again demonstrate that patients differ considerably, one from another, in their response to analgesics. Since this has been a consistent finding in our studies, it underscores the need for having each patient serve as his own control (cross-over design). If this had not been a cross-over study, it is estimated on the basis of the large variation among patients that we would require about four times as much data to obtain the same confidence in our results.

The orthogonal comparisons[8] show the extent to which the morphine and aspirin effects contributed to the treatment variance and serve as measures of the significance of their effects (Table II). The factorial design of the experiment provides, in fact, two estimates of the effects of each drug. For example, the scores of morphine minus the placebo, and of the combination minus aspirin, are both measures of morphine effect. In addition, one may obtain an estimate of the interactions of the drugs as is shown in Table II. The effects of morphine and of aspirin were found to be statistically significant, while that of the interaction of the two drugs was not.

The significantly greater effect with aspirin than with a placebo is a further indication of the sensitivity and validity of the

Table II. *Factorial analysis of effect*

	Placebo	*Aspirin*	*Morphine*	*ASA + MS*	*Mean square*	*F ratio*
Capsules P.O.	Lactose	ASA–600 mg.	Lactose	ASA–600 mg.		
Injection I.M.	Saline	Saline	MS–10 mg.	MS–10 mg.		
Cumulative scores	80	130	242	290		
Aspirin	−	+	−	+	54.6	7.01*
Morphine	−	−	+	+	587.8	75.4 *
Interaction	+	−	−	+	0.02	0.003

Assay error: combined interactions: 7.8.

*Significance level: $p < 0.01$.

Table III. *Analgesic reponses to standard medication and placebo. Mean pain relief scores*

Study	*No. patients*	*No. doses*	*MS 10 mg.*	*Placebo*		*ASA 600 mg.*
				Saline	*Lactose*	
I	12	24	4.79	2.08		
II	10	20			2.50	4.20
III	16	24	3.66	1.96		
IV	16	29			0.97	2.35
V	20	27	3.76	1.96		
VI	16	33			0.82	3.90
VII	10	17	7.47	3.76		
VIII	19	38			3.10	4.96
IX	20	33	6.73	3.00		
X	14	28			2.14	4.04
XI	20	32			3.00	5.67
XII	11	20			2.20	4.75
XIII	28	45	5.35	3.15		
XIV	14	23			1.44	5.30
XV	28	44	5.50	– 1.82 –		2.95
XVI	17	27			4.04	4.71
XVII	18	32	5.00	1.13		
XVIII	18	30			3.57	4.37
XIX	27	44			1.89	4.04
XX	27	44			2.64	4.50
XXI	14	26	4.46	1.69		
XXII	15	22	8.27	4.83		
XXIII	31	50	5.12	1.96		
XXIV	27	46	5.46	2.33		
XXV	34	61	6.49	3.13		
XXVI	8	13	9.07	3.00		
XXVII	6	9			2.44	5.00
XXVIII	18	30			4.00	6.37
XXIX	27	47			2.91	4.14
XXX	13	24			2.58	6.00
XXXI	18	31			2.58	4.55
XXXII	39	63			3.43	4.05
XXXIII	18	31			2.83	5.19
XXXIV	11	17			2.29	5.29

method of assay. We cannot conceive that these patients could possibly have reported more pain relief from aspirin simply on the basis of being able to recognize pleasurable side effects of this drug under the conditions of this study.

The therapeutic implications of the results are that aspirin is an effective analgesic in patients with pain due to cancer (though in oral 600 mg. doses it is significantly less effective than 10 mg. of morphine sulfate given intramuscularly) and that by administering it together with morphine, one may expect to obtain significantly greater pain relief than by giving morphine alone. The pharmacologic implications of the results are that the drugs appear to act independently of one another in producing analgesia, for the small and insignificant interaction value is an indication that they neither interfere with nor enhance the analgesic effect of one another. This suggests that the sites or modes of action of aspirin and morphine are different.

Morphine and aspirin as controls. Table III lists the mean total pain relief scores obtained with intramuscular injections of 10 mg. morphine sulfate and saline, and with oral doses of 600 mg. aspirin and lactose in 34 chronologic studies in which these medications served as reference standards and placebo controls in evalua-

tion of other drugs for analgesic effects. As may be noted, all of these studies were carried out in relatively small groups of patients, and the discrepancy between the number of doses and the number of patients in each study simply reflects the fact that some patients received each medication twice and others only once. Study XV is the aspirin and morphine study reported above, and here the placebo consisted of both intramuscular saline and oral lactose.

The over-all mean score for 10 mg. of morphine sulfate given intramuscularly was 5.64 categories of relief, and for its corresponding saline placebo 2.49. For 600 mg. aspirin by mouth the mean score was 4.47, and for its lactose placebo 2.58. However, the ranges of scores for morphine (3.66-9.07) and its corresponding placebo (1.13-6.37), and for aspirin (2.35-6.37) and its placebo control (0.82-4.04) show considerable overlap and are an indication that the actual or absolute scores in themselves have little meaning. The more important statistics are that different patient groups varied considerably in their levels of response to these standard and placebo medications, and yet in each study the standard drug was significantly superior to its placebo control. In fact, it is only by the use of such internal controls in each study[6] that we have any reassurance that our sample patient-population can distinguish an analgesic from an inert placebo.

Discussion

A method for evaluating analgesic drugs in patients with pain, that basically involves merely asking the patient how much pain he has, is capable of producing valid quantitative information on drug action. Such parameters of analgesia as peak action, onset, duration, the time-effect curve, and an estimate of the area under the curve can be obtained by this simple technique. With the use of appropriate experimental control, the method has proved capable of discriminating between the effect of known analgesics (morphine and aspirin) and that of a placebo and is indeed sensitive enough to distinguish between levels of effect of different analgesic drugs (morphine, aspirin, and morphine plus aspirin; graded doses of morphine).

Certainly there is nothing novel in utilizing controls in the design of an experiment although only a few investigators of analgesics have thought fit to apply them.[1] The pain chart also is not a new device, having been reported by Keele[5] in England in 1948. The contribution of this work, we feel, lies in the demonstration that the pain chart can be utilized as a measuring tool in controlled studies. The data can be interpreted in terms of quantitative scores of pain relief which prove to be sensitive measures of a variety of parameters of analgesic effect and are in a form suitable for analysis by standard statistical techniques.

It is well to keep in mind that, despite their sensitivity to the analgesic effects of drugs, the pain relief scores are at best only rough measures, approximations of what is going on. What do pain relief scores of, say, 4 or 8 points tell us about the potency of the drug on the one hand and the state of comfort of the patient on the other? To answer this, it is necessary to reexamine the categories of pain severity from which these scores are derived. These categories are gross, arbitrary, and highly subjective measures of intensity of an experience which differs profoundly from one patient to another. Although the categories are all weighted equally in measuring hourly pain relief, there is no way of determining whether they represent linear increments of pain to the patient or indeed whether or not they mean different things to different patients. The highly significant variation among patients consistently seen in our studies appears to indicate that at least this latter point is true. A linear scale was decided upon simply in that, without evidence to the contrary, it seemed logical to use the simplest scale. In these gross measurements, a refinement would be somewhat of a presumption.

It must also be kept in mind that some of the data are diluted when pain becomes

greater than the premedication level or when an additional analgesic is administered in less than 6 hours after the test drug. In both of these cases hourly scores of zero relief are arbitrarily assigned and it is indeed possible that this may not adequately reflect the status of the patient at the time. Mosteller[7] has correctly pointed out that random fluctuations in the degree of pain go both up and down. However, utilizing negative relief scores presents what to us is an insoluble problem—how to score the remaining hours in patients who have received a second analgesic in less than 6 hours after the test drug. Would the pain have risen above or remained at the premedication level had no other drug been given? Since this cannot be resolved satisfactorily, an arbitrary, universal cutoff point at zero relief was decided upon. The problem of negative relief can occur only when the premedication pain level is not *severe pain.* Since this represents roughly only about one fifth of our data, the effect of the arbitrary zero cutoff is a minor one.

Despite the variations between patients and the limitations of the scale, pain relief scores are not without advantages. They exhibit a considerable degree of consistency within each patient from one occasion to the next. This can be seen in the small variation between replications and in the low mean square values for the patient by replication and treatment by replication interactions. It would appear that each patient finds his own level on the scale and reacts more or less consistently at this level. As can be seen from the scatter diagram for morphine and saline, this level will vary considerably from one patient to the next. An individual may make errors of drug discrimination within his level of response, but the group is able to distinguish even graded doses of drug.

At this point, let us attempt to answer the question of the meanings of the scores. By their very nature these are not absolute measures of analgesia or level of patient comfort, but highly personal, individualized subjective responses. A score of 4 or 8 points cannot be interpreted as an absolute measure of drug potency; witness the variation in responses to morphine and aspirin in Table III. A closer examination of this table, however, will show that, despite these variations, the relative responses of these drugs and their corresponding placebos are remarkably consistent. Scores of 4 and 8 points then, within a study in the same population represent a meaningful *relative* comparison of drug effects. Projections of scores from one small population group to another, however, have extremely limited meaning. In terms of the individual patient as well, scores must be interpreted in the light of his own level of response. A score of 4 points may be an adequate level of analgesia for one patient and no more than a placebo effect for another.

We have employed standard statistical tests of significance (analysis of variance, Student's t test) to determine the level of confidence that we can have in our data. Indeed the random, uncontrolled variations that occur in the clinical situation dictate that a difference between treatments must be found significant by some statistical test before it can be accepted as a true drug effect.[6] Are the statistical tools developed for normally distributed, quantitative information applicable to our plain relief scores? Our data are quasi-quantitative (based on a limited scale of categories of intensity) and a study of the scatter diagram (Fig. 3) will show that the pain relief scores for both morphine and placebo have skewed distributions, the majority being grouped at the lower end of the scale with a few responses trailing off toward the higher scores. Skewing of this sort will somewhat affect the significance level of the data, so that an apparent probability of 5 per cent may actually be slightly less or more, but valid comparisons can still be made. A wide variety of conversions (square root of the scores, logarithm of scores, and others) do not adequately correct the skewing or alter the results or significance levels. Nonparametric, rank order tests, such as that devised by Wilcoxon,[10] which make no as-

sumptions as to distribution of the data, yield significance levels similar to those obtained in the analysis of variance. These nonparametric tests lose somewhat in sensitivity by substituting rank order for quantitative scores and tell us none of the important information about subgroup variations and interactions that can be obtained from analysis of variance. We have chosen to accept a small possible error in terms of significance levels as the cost of obtaining greater information about our studies, and routinely perform analysis of variance on our data.

The universal prevalence of placebo reaction in our studies has made academic, in our minds, at least, the issue raised by some investigators[2] of eliminating placebo reactors from studies. Since a large pr portion of placebo reactors do discrim in terms of intensity of effect, the mo ertinent problem is the number of non rimiinators in our studies. We have nc of eliminating them, expect in retrospe nd this would be a dubious practice at best. Every patient, discriminator or not, who completes a cross-over drug comparison is included in the results. The length of a study will then depend on the proportion of discriminators in the particular population and the magnitude of drug differences being studied.

The design of the study will be dictated by the type of information one hopes to obtain. The experimental design of the morphine and aspirin study illustrated here is appropriate for assaying for the presence or absence of analgesic effect, or for studying the effects of combinations of drugs. Assaying for an estimate of relative potency, for example, would require a considerably altered experimental design.

Assaying for the presence or absence of analgesics is merely a first, but basic, step in the clinical evaluation of an analgesic drug. The approach that we have developed has been influenced to no small extent by the fact that we are dealing with the problems of patients with chronic pain. The use of total pain relief scores, or even possibly of completely cross-over studies might be of considerably less moment to an investigator concerned with postoperative pain. In any event, we hope that, by our method, we are able to obtain data that can be profitably compared with those of other investigators doing controlled studies under different conditions.

The authors wish to express their indebtedness to the professional and administrative staffs of the Memorial Center, and in particular of the James Ewing Hospital, for their cooperation and assistance in these studies, and to the late Dr. Cornelius P. Rhoads and to Dr. Rulon Rawson for their encouragement and support of this program. Debts of gratitude are also due to Drs. John C. Seed and Irwin Bross, and Mrs. Marisa Mihich, for advice and assistance in the design and statistical analysis of these experiments, and to Miss Julie Franchi for assistance in the preparation of this manuscript.

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Evaluation of 3-benzylthiomethyl chlorothiazide
A new oral diuretic

A clinical evaluation is presented of the relative diuretic effectiveness of benzylthiomethyl chlorothiazide and hydrochlorothiazide in 17 edematous patients. Comparing single 100 mg. doses of each drug, benzylthiomethyl chlorothiazide is about 85 per cent as effective as hydrochlorothiazide.

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This study was undertaken to compare clinically the diuretic response resulting from benzylthiomethyl chlorothiazide, a newer substituted benzothiadiazine, with that obtained from chlorothiazide. The effect of each agent on body weight, urine volume, and urine sodium and potassium contents was observed. Patients received, in random order, one of these drugs or a placebo on each day of a consecutive 3 day period. All subjects were hospitalized; all had fluid retention meriting diuretic therapy. Seventeen patients were studied (7 had organic heart disease with congestive heart failure, 5 hepatic cirrhosis, 2 the nephrotic syndrome associated with diabetic nephropathy, 2 lymphoblastoma, and one idiopathic cyclical edema).

Methods

A single oral dose of either 100 mg. hydrochlorothiazide or 100 mg. benzylthiomethyl chlorothiazide, or a placebo tablet of identical size and shape, was given to each patient at 7:00 A.M. The amount of drug and the frequency of its administration were chosen for comparability rather than for maximal diuretic effectiveness. The composition of any tablet used was known neither by the physician giving it nor by the subject receiving it. The order of administration of the three substances during the 3 day period was randomized to compensate for any effect that one agent might have on the action of a second given the following morning.

Each patient was given any additional necessary medication and had the same diet prescribed for at least 5 days prior to and during the 3 days of administration of the diuretic drugs, so that these factors would not affect this comparative study. Two subjects received a regular hospital diet, 5 a 200 mg. sodium diet, and 10 an 800 mg. sodium diet daily. Seven patients were maintained on 0.1 Gm. digitalis daily. One patient received 1 Gm. potassium chloride 3 times each day.

Twenty-four hour urine collections were divided into the first 6 hours after the drug was given (7:00 A.M. to 1:00 P.M.) and the

Diuretic drugs supplied were 3-benzylthiomethyl chlorothiazide (3-benzylthiomethyl-6-chloro-7-sulfamyl-1,2,4,-benzothiadiazine-1, 1-dioxide) as P-1393 by Chas. Pfizer & Co., Inc., and hydrochlorothiazide (6-chloro-7-sulfamyl-3, 4-dihydro-1,2,4-benzothiadiazine-1, 1-dioxide) as Hydrodiuril by Merck Sharp & Dohme Research Laboratories.

Table I. *Urine volume, sodium and potassium excretion after a placebo, benzylthiomethyl chlorothiazide, and hydrochlorothiazide*

Patient No.	*Diagnosis*	*Drug given*	*0 to 6 hours*			*6 to 24 hours*		
			Urine volume (ml.)	*Sodium content (mEq.)*	*Potassium content (mEq.)*	*Urine volume (ml.)*	*Sodium content (mEq.)*	*Potassium content (mEq.)*
1	Heart failure	Placebo	256	1.2	7.6	550	2.8	24.4
		BTM*	310	0.7	24.4	653	9.5	54.6
		Hydro†	132	11.1	2.0	580	8.8	48.0
2	Lymphoma	Placebo	440	3.6	16.7	560	3.8	22.2
		BTM	470	3.1	14.1	753	6.5	35.5
		Hydro	610	7.2	26.7	400	3.1	15.4
3	Hepatic cirrhosis	Placebo	280	3.2	14.8	210	1.4	7.7
		BTM	74	0.7	4.4	375	2.6	16.6
		Hydro	420	19.8	24.6	810	24.1	47.5
4	Hepatic cirrhosis	Placebo	340	2.6	24.8	400	3.0	25.7
		BTM	160	1.3	13.3	460	3.5	34.1
		Hydro	295	2.2	14.8	440	2.9	20.1
5	Heart failure	Placebo	170	1.7	15.3	400	4.5	40.6
		BTM	550	56.4	32.9	725	6.6	46.6
		Hydro	490	5.8	43.5	980	13.3	94.0
6	Heart failure	Placebo	160	6.8	15.1	1,210	11.1	53.7
		BTM	85	2.1	7.3	1,470	16.5	62.8
		Hydro	210	10.3	19.0	1,600	39.0	68.0
7	Lymphoma	Placebo	46	3.3	8.7	540	72.7	78.0
		BTM	130	19.5	21.4	415	63.1	62.2
		Hydro	95	13.7	16.5	200	38.3	32.7
8	Hepatic cirrhosis	Placebo	190	14.7	13.4	390	32.4	36.3
		BTM	170	26.6	13.8	720	129.6	56.7
		Hydro	320	73.5	28.0	860	203.2	48.0
9	Hepatic cirrhosis	Placebo	510	36.8	14.5	230	29.3	9.4
		BTM	260	19.9	19.9	375	24.1	16.8
		Hydro	440	62.3	13.5	585	49.8	35.1
10	Diabetic nephropathy	Placebo	235	5.9	12.0	1,160	12.8	74.6
		BTM	310	12.3	20.7	1,250	24.9	104.9
		Hydro	480	20.9	63.5	1,955	47.7	134.8
11	Heart failure	Placebo	510	2.8	25.0	1,375	8.4	63.3
		BTM	780	10.2	55.1	774	82.1	10.6
		Hydro	168	3.3	23.5	1,124	17.5	103.4
12	Heart failure	Placebo	280	14.6	6.9	940	7.0	23.9
		BTM	218	2.0	18.7	950	9.8	65.0
		Hydro	580	52.6	60.3	1,980	101.3	105.0
13	Hepatic cirrhosis	Placebo	555	74.9	26.0	378	34.9	28.7
		BTM	400	50.7	29.6	688	49.4	38.5
		Hydro	48	5.8	2.9	510	30.2	34.4
14	Heart failure	Placebo	165	3.1	16.8	980	75.7	98.0
		BTM	230	11.9	23.0	135	5.8	16.5
		Hydro	200	15.0	20.0	740	29.0	68.5

Table I
Continued

Patient No.	Diagnosis	Drug given	0 to 6 hours			6 to 24 hours		
			Urine volume (ml.)	Sodium content (mEq.)	Potassium content (mEq.)	Urine volume (ml.)	Sodium content (mEq.)	Potassium content (mEq.)
15	Cyclical edema	Placebo	410	7.5	11.3	1,500	22.8	87.7
		BTM	705	40.2	32.3	855	30.6	43.3
		Hydro	1,318	65.9	27.6	1,230	113.7	55.9
16	Heart failure	Placebo	700	37.7	9.2	588	57.4	14.5
		BTM	410	55.4	12.7	1,350	109.9	39.3
		Hydro	715	62.4	26.1	380	17.1	6.2
17	Diabetic nephropathy	Placebo	645	17.8	32.3	1,950	17.0	73.0
		BTM	1,540	91.8	42.7	2,400	133.4	90.2
		Hydro	1,550	89.9	62.0	1,930	48.3	65.4
Mean		Placebo	346±70	14.0±4.7	15.9±1.7	785±46	23.4± 5.9	44.8±6.7
±		BTM	402±29	23.8±6.3	22.7±3.1	848±41	41.6±11.0	46.7±6.2
S.E.M.‡		Hydro	475±39	30.7±7.1	27.9±4.5	959±45	46.3±12.3	57.8±8.5

*BTM, 100 mg. benzylthiomethyl chlorothiazide.
†Hydro, 100 mg. hydrochlorothiazide.
‡S.E.M. = Standard error of mean.

subsequent 18 hours (1:00 P.M. to 7:00 A.M. the following morning). Body weight was recorded at 7:00 A.M. each day, after voiding and prior to administration of drug. Urine volumes were measured and sodium and potassium contents determined by flame photometry.

Results

Calculated mean urine volumes and mean sodium and potassium excretions of all patients following administration of each diuretic agent and the placebo are shown in Fig. 1. Values determined for individual patients are shown in Table I. In comparison with the mean 24 hour urinary output observed with the placebo (1,131 ml.), benzylthiomethyl chlorothiazide increased mean urine volume by 119 ml., whereas hydrochlorothiazide induced a 303 ml. increase in the same period of time. The diuresis resulting from both hydrochlorothiazide and benzylthiomethyl chlorothiazide was greater within the first 6 hours than in the following 18 hours. The mean 24-hour weight loss was 1.04 pounds after benzylthiomethyl chlorothiazide, 1.12 pounds after hydrochlorothiazide, and 0.58 pound after placebo.

Hydrochlorothiazide caused more natriuresis and kaliuresis than did benzylthiomethyl chlorothiazide in the doses used. In the first 6 hours it increased mean sodium excretion 119 per cent and potassium excretion 76 per cent above control values. The accompanying increase in urine volume was 37 per cent. In the subsequent 18 hours sodium output increased 98 per cent, potassium 29 per cent, and urine volume 22 per cent. During entire 24 hours after hydrochlorothiazide as given the total sodium excretion was 103 per cent greater, total potassium 41 per cent greater, and urine volume 27 per cent greater than the mean values during the 24 hours following placebo administration.

During the first 6 hours after benzylthiomethyl chlorothiazide was given, sodium

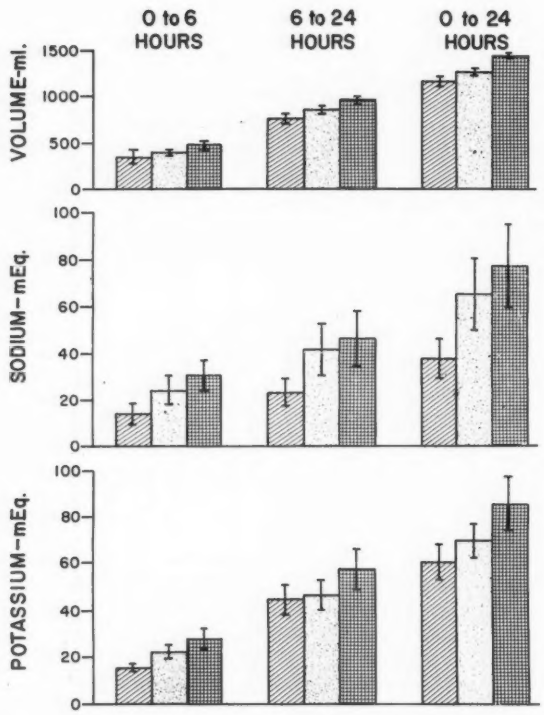

Fig. 1. The mean total urine volume, sodium, and potassium excreted in the first 6 hour period (on the left), the subsequent 18 hour period (in the center), and the entire 24 hour period (on the right) after a placebo tablet (diagonal line column), 100 mg. 3-benzylthiomethyl chlorothiazide (white column), and 100 mg. hydrochlorothiazide (column of squares). Vertical lines represent range of standard error of the mean.

excretion rose 70 per cent, potassium output 46 per cent, and urine volume 16 per cent above the mean control values. The next 18 hours showed a sodium increase of 78 per cent, potassium of 4 per cent, and a volume rise of 8 per cent. In the entire 24 hours after benzylthiomethyl chlorothiazide administration, total sodium excretion increased 75 per cent, total potassium 14 per cent, and urine volume 12 per cent above the mean levels found when the placebo was given.

These percentages are not absolute values as they are derived from means which, as shown in Fig. 1, have considerable ranges of standard error. They do afford a relative comparison of the two diuretic agents, however. If one directly compares the efficacy of these diuretic drugs, it is found that in the first 6 hours after benzylthiomethyl chlorothiazide administration, sodium excretion was 77 per cent of that seen after hydrochlorothiazide, potassium was 81 per cent, and urine volume was 85 per cent. During the subsequent 18 hours the comparable figure for sodium excretion was 90 per cent, potassium 81 per cent, and urine volume 88 per cent. In the entire 24 hour period after benzylthiomethyl chlorothiazide was given there was 85 per cent as much sodium, 81 per cent as much potassium, and 87 per cent of the volume of urine excreted as there was after hydrochlorothiazide administration.

Individual patients showed varying responses to these agents. Nine patients exhibited greater volume diuresis with hydrochlorothiazide; in 2 of these benzylthiomethyl chlorothiazide had less effect than the placebo. Five subjects showed a greater diuretic response with benzylthiomethyl chlorothiazide; in 3 of these hydrochlorothiazide effected less response than the placebo. Three patients had little increase in urine volume after receiving either drug. Eleven patients demonstrated a greater natriuretic and kaliuretic effect of hydrochlorothiazide; in 3 of these there was less sodium and potassium excretion after benzylthiomethyl chlorothiazide than after the placebo. Four subjects eliminated more sodium and potassium after receiving benzylthiomethyl chlorothiazide; 2 of these excreted less sodium and potassium after hydrochlorothiazide than after the placebo. Two patients were relatively unresponsive, in respect to excretion of these ions, to the administration of both drugs. It should be emphasized that there was no correlation between the effectiveness of a particular agent and a specific disease process. No side effects from these drugs were observed in any of these patients.

Discussion

Administration of a placebo provides a control day during each 3 day period and allows evaluation of the effect of hydrochlorothiazide and benzylthiomethyl chlorothiazide on urine volume and electrolyte

excretion in each patient in relation to that seen when no diuretic agent is given. Since hydrochlorothiazide is a drug of known diuretic potency, it serves as a standard against which to judge benzylthiomethyl chlorothiazide.[1,2] On a weight basis hydrochlorothiazide is 10 to 12 times as potent a diuretic agent as chlorothiazide; however, it is only one ninth as potent a carbonic anhydrase inhibitor.[3] Its greater diuretic potency is due to a greater mercury-like effect. Benzylthiomethyl chlorothiazide also shows an electrolyte excretion pattern analogous to that of the mercurial diuretics.*

In evaluating a diuretic agent the most significant factor is its natriuretic effect, since sodium is the principal ionic constituent of expanded extracellular volume. Sodium loss is necessary for the mobilization of edema fluid. On the other hand, potassium loss is not desirable. A specific danger of potassium loss is the potentiation of digitalis toxicity. This has been reported during chlorothiazide therapy.[4] The optimal objectives of diuretic therapy therefore are maximal sodium loss accompanied by minimal potassium loss.

From the data presented here, based on dosage levels employed and the conditions of this study, hydrochlorothiazide causes a greater increase in urine volume and more natriuresis and kaliuresis than does benzylthiomethyl chlorothiazide. However, benzylthiomethyl chlorothiazide is nearly as effective in its natriuretic and diuretic actions, being about 85 per cent as potent as is hydrochlorothiazide. The kaliuretic effect of benzylthiomethyl chlorothiazide was slightly less, although of similar magnitude, being 81 per cent of that caused by hydrochlorothiazide. In so far as these electrolytes are concerned, it appears that these drugs induce comparable excretory patterns.

*Based on information supplied by Dr. D. G. Iezzoni, Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

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Further studies on the evaluation of antitussive agents employing experimentally induced cough in human subjects

The feasibility of employing experimental cough induced by citric acid aerosols as a quantitative method for assessing antitussive activity is confirmed in this study. Thirteen coded drugs were tested by the double blind technique on trained healthy subjects. Differences in both degree and duration of effect on the cough response as recorded by pneumotachographic tracings permitted the grouping of these agents into three general categories: ineffective drugs, preparations which had sustained antitussive activity over the 4 hour test period, and those with maximal cough suppression at the second hour with a waning of effect thereafter. Mechanisms responsible for these differences are discussed.

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The present report is an extension of studies on the evaluation of antitussive agents employing experimentally induced cough in human subjects. In previous studies[1,2] on the methodology of producing cough, the inhalation of citric acid aerosols was shown to elicit a consistent cough response in healthy subjects who were challenged with the irritant at hourly intervals over a 4 hour period. In order properly to assess unknown drugs by the double blind technique with this method, certain criteria with respect to the subject material were essential.

A total of 16 healthy technicians and research fellows (8 men and 8 women) with a mean age of 34 years participated in these studies. These individuals have been employed in previous antitussive studies during the past 5 years and represent a "trained" subject pool. By repeated exposure to varying concentrations of citric acid aerosol, the "threshold" level necessary to provoke cough has been determined in each individual. Although the threshold concentration of irritant varied from subject to subject, it has remained remarkably stable in the same individual from hour to

This study has been supported by grants from Merck & Co., Rahway, N. J., The Upjohn Company, Kalamazoo, Mich., Ciba Pharmaceutical Products, Inc., Summit, N. J., R. J. Strasenburgh Co., Rochester, N. Y., and the Bristol-Myers Co., N. Y.

Table I. *Drugs tested by double blind technique*

Code	*Drug*	*Pharmaceutical designation*	*Dose (mg.)*
X	Placebo	79 C	—
A′	Placebo	11409–RJS	—
E′	Placebo	39–EU	—
C	N-methylhomopiperonylamine	Homarylamine	10
F			20
L			40
U	Morpholiny[illegible]ylmorphine	Morphinolinylethylmorphine	10
Y	β methyl-α, α diphenyl-1-piperidine ethanol HCl	4964-U	30
W			60
Z	ω-methoxypoly (ethyleneoxy)-ethyl *p*-butylaminobenzoate	Benzonatrate	100
B′	Dihydrocodeinone resin complex	Dihydrocodeinone resin	5
C′	6 dimethylamino-4,4 diphenyl-3-hexanone HCl	9558-U	7.5
D′	dl-4,4-diphenyl-6-dimethyl-amino-3-heptanone	Methadone	2.5

hour over years of testing. Nevertheless, this level was redetermined for each subject prior to participation in a "test run." The concentrations of citric acid representing the threshold stimulus for the subjects in the present study were as follows: 1 at 25 per cent, 1 at 20 per cent, 2 at 15 per cent, 7 at 10 per cent, 3 at 5 per cent, and 2 at 2.5 per cent.

The thirteen drugs submitted for evaluation were coded alphabetically and administered to each subject in random sequence as identical white capsules with the use of a double blind technique. When these agents were decoded at the completion of the study, three of the preparations were found to be placebos. On the basis of pharmacologic properties, the remaining drugs could be separated into the following groups:

1. Morphinoliny[illegible]hylmorphine,* dihydrocodeinone res[illegible]† methadone, and 9558-U‡ are narc[illegible]gents. They possess analgesic activity [illegible]quivalent to the codeine-morphine drugs and are respiratory depressants.

2. Homarylamine and 4964-U are chemically related to the sympathomimetic amines. They exhibit weak anticholinergic and antihistaminic activities but have no analgesic properties.

3. Benzonatrate* is a congener of tetracaine with local anesthetic properties. At the dosage level employed in this study, it is not a central respiratory depressant.

The various agents together with their dosages and code designations are presented in Table I.

Methods

The instrumentation and procedure employed to elicit cough with citric acid aerosols have been described in detail in a previous paper.[1] Graphic tracings of the cough response elicited by the aerosol are obtained with a pneumotachograph and an Altec condenser microphone, and record the peak and mean flow rates of air expelled with cough and the "sound pressure" wave, respectively. The frequency, or number of coughs per stimulus, may be determined from either the microphone or

*Pholcodeine.
†Tussionex.
‡Ticarda.

*Tessalon.

Table II. *Antitussive effect of drugs on the frequency of cough elicited by citric acid aerosol in healthy subjects*

Drug	*Tests per hour*	*Cough interval*	*Mean % change from control*	*Confidence limits (95%)**
X	50	Control		±25.5
(Placebo)		1 hour	−11.3	NS†
		2 hours	− 1.3	NS
		3 hours	+ 3.8	NS
		4 hours	0	NS
A′	50	Control		±18.0
(Placebo)		1 hour	0	NS
		2 hours	− 7.1	NS
		3 hours	+11.0	NS
		4 hours	+11.8	NS
E′	50	Control		±23.0
(Placebo)		1 hour	−25.4	S‡
		2 hours	− 5.3	NS
		3 hours	+ 2.6	NS
		4 hours	− 1.8	NS
C	35	Control		±28.1
(Homarylamine)		1 hour	−23.2	NS
10 mg.		2 hours	−28.0	NS
		3 hours	−19.5	NS
		4 hours	− 4.9	NS
F	27	Control		±19.2
(Homarylamine)		1 hour	−23.2	S
20 mg.		2 hours	−42.0	S
		3 hours	−17.4	NS
		4 hours	−13.0	NS
L	36	Control		±20.1
(Homarylamine)		1 hour	−16.0	NS
40 mg.		2 hours	−26.0	S
		3 hours	−32.0	S
		4 hours	−24.0	S

*Three-factor analysis of variance (subject-hour-order) expressed as 95 per cent limit.
†Not significant.
‡Significant.

pneumotachographic tracings.

On the day of a test, subjects were selected who presented no evidence of throat irritation or a recent upper respiratory infection. Each individual had been previously cautioned against taking any medication with known or suspected antitussive activity. A control study was obtained in response to five inhalations of the appropriate concentration of citric acid aerosol with a rest period of 1 to 2 minutes between each inhalation. During this rest period, a few sips of water were swallowed to remove any residue of citric acid which might have run down upon the tongue or oropharynx. Immediately after this control "run," the drug to be tested was administered orally and studies were performed at hourly intervals for a period of 4 hours. With the same procedure, two preparations, A′ placebo and B′ dihydrocodeinone resin, were retested over a 7 hour period.

Results

From previous studies, the most appropriate indices for assessing the antitussive

Table II.
Continued

Drug	*Tests per hour*	*Cough interval*	*Mean % change from control*	*Confidence limits (95%)**
U	60	Control		±13.6
(Morphinolinylethylmorphine)		1 hour	−30.4	S
10 mg.		2 hours	−27.4	S
		3 hours	−26.8	S
		4 hours	−23.8	S
Y	60	Control		±24.2
(4964–U)		1 hour	−32.7	S
30 mg.		2 hours	−20.7	NS
		3 hours	−21.2	NS
		4 hours	+12.0	NS
W	55	Control		±23.6
(4964–U)		1 hour	−30.0	S
60 mg.		2 hours	−29.2	S
		3 hours	−34.3	S
		4 hours	−33.1	S
Z	40	Control		±20.3
(Benzonatrate)		1 hour	−26.6	S
100 mg.		2 hours	−35.4	S
		3 hours	−20.3	S
		4 hours	−24.0	S
B′	50	Control		±21.2
(Dihydrocodeinone resin)		1 hour	−36.3	S
5 mg.		2 hours	−31.6	S
		3 hours	−23.5	S
		4 hours	−26.5	S
C′	50	Control		±23.4
(9558–U)		1 hour	− 7.0	NS
7.5 mg.		2 hours	− 6.1	NS
		3 hours	+14.8	NS
		4 hours	+20.9	NS
D′	50	Control		±23.8
(Methadone)		1 hour	−34.4	S
2.5 mg.		2 hours	−38.2	S
		3 hours	−21.0	NS
		4 hours	−17.6	NS

activity of these drugs appeared to be the decrease in the number of coughs per stimulus (frequency) from the control value and the reduction in the product of the mean flow rate and volume expelled for all coughs elicited by each inhalation of citric acid. The latter served as a relative measure of cough intensity or effort. Data for cough "frequency and intensity" were obtained in response to a total of 750 inhalations of citric acid aerosol for the three drugs of the placebo series; 490 for the homarylamine series; 575 for the 4964-U series; 300 for morphinolinylethylmorphine; 200 for benzonatrate, and 250 each for dihydrocodeinone resin, 9558-U, and methadone. An additional 280 and 400 inhalations of citric acid aerosol were employed for A′ placebo and dihydrocodeinone resin, respectively, in the 7 hour retest study.

Measurements derived from the graphic tracings of the cough responses elicited by a total of 3,745 inhalations have been subjected to statistical analyses by use of the 3 factor analysis of variance in which the

Table III. *Antitussive effect of drugs on the expiratory air flow of induced cough (total mean flow rate in liters per second)*

Drug	*Tests per hour*	*Cough interval*	*Mean % change from control*	*Confidence limits (95%)**
X	50	Control		±24.2
(Placebo)		1 hour	− 1.9	NS
		2 hours	+ 4.5	NS
		3 hours	+ 3.4	NS
		4 hours	+ 4.5	NS
A′	50	Control		±13.5
(Placebo)		1 hour	+ 3.8	NS
		2 hours	− 1.7	NS
		3 hours	+ 1.2	NS
		4 hours	+ 4.1	NS
E′	50	Control		±19.5
(Placebo)		1 hour	−21.4	S
		2 hours	− 8.8	NS
		3 hours	−10.0	NS
		4 hours	−13.9	NS
C	35	Control		±24.0
(Homarylamine)		1 hour	−21.9	NS
10 mg.		2 hours	−27.4	S
		3 hours	−20.0	NS
		4 hours	−12.1	NS
F	27	Control		±20.4
(Homarylamine)		1 hour	−27.0	S
20 mg.		2 hours	−45.0	S
		3 hours	−26.8	S
		4 hours	−20.8	S
L	36	Control		±19.8
(Homarylamine)		1 hour	−13.3	NS
40 mg.		2 hours	−25.6	S
		3 hours	−34.1	S
		4 hours	−15.9	NS

*Three-factor analysis of variance (subject-hour-order) expressed as 95 per cent limit.

subject, hour, and order of stimulus are integrated.[3] Statistical significance for each drug has been indicated at the 95 per cent confidence limit by conversion of the F-values into a percentile variation about the mean per cent changes from the control level for each hourly period. Thus any effect resulting from chance would occur less than one time in twenty.

The effect of the various drugs on the "frequency" of coughing is shown in Table II.

Placebos. It is apparent that except for the first hour response to E′, none of the placebos showed any significant suppression in the frequency of cough over the 4 hour test period. In a review of the data for each of the subjects tested against E′, it was noted that one subject had a marked reduction in the number of coughs from 18 to 6 at the first hour period. This cannot be explained; but if we were to correct for this single discrepancy, the mean per cent change for the first hour would be 17 per cent.

Homarylamine. Although the 10 mg. dose of homarylamine did not appear to be significant, the subjects exhibited consid-

Table III
Continued

Drug	*Tests per hour*	*Cough interval*	*Mean % change from control*	*Confidence limits (95%)**
U	60	Control		±14.8
(Morphinolinylethylmorphine)		1 hour	−26.8	S
10 mg.		2 hours	−26.6	S
		3 hours	−26.3	S
		4 hours	−20.9	S
Y	60	Control		±21.6
(4964–U)		1 hour	−28.8	S
30 mg.		2 hours	−19.8	NS
		3 hours	−23.9	S
		4 hours	− 3.5	NS
W	55	Control		±19.2
(4964–U)		1 hour	−30.4	S
60 mg.		2 hours	−31.0	S
		3 hours	−34.4	S
		4 hours	−31.0	S
Z	40	Control		±21.7
(Benzonatrate)		1 hour	−21.7	S
100 mg.		2 hours	−28.0	S
		3 hours	−17.5	NS
		4 hours	−14.4	NS
B′	50	Control		±18.8
(Dihydrocodeinone resin)		1 hour	−33.6	S
5 mg.		2 hours	−34.5	S
		3 hours	−27.4	S
		4 hours	−23.8	S
C′	50	Control		±22.0
(9558–U)		1 hour	− 1.3	NS
7.5 mg.		2 hours	− 7.1	NS
		3 hours	+ 4.5	NS
		4 hours	+ 0.9	NS
D′	50	Control		±21.7
(Methadone)		1 hour	−30.3	S
2.5 mg.		2 hours	−36.3	S
		3 hours	−30.0	S
		4 hours	−20.6	NS

erable variation in response as evidenced by the high confidence limit. The 20 and 40 mg. doses were effective for 2 and 3 hours, respectively.

Morphinolinylethylmorphine. A 10 mg. dose showed significant cough suppression over the 4 hour test.

4964-U. The 30 mg. dose of 4964-U was significant for the first hour, whereas the 60 mg. dose was clearly significant over the entire test period.

Benzonatrate. A dose of 100 mg. demonstrated significant cough suppression over the 4 hour test.

Dihydrocodeinone resin. Effective antitussive activity was evident during the 4 hour test period following administration of dihydrocodeinone resin. The 7 hour study will be discussed below.

9558-U. This drug was ineffective in suppressing cough and behaved in most respects like the placebos.

Methadone. Methadone, 2.5 mg., exhibited a considerable decrease in cough frequency over the first 2 hours but the antitussive effect appeared to wane over the third and fourth hours.

In general, the effect of these agents on

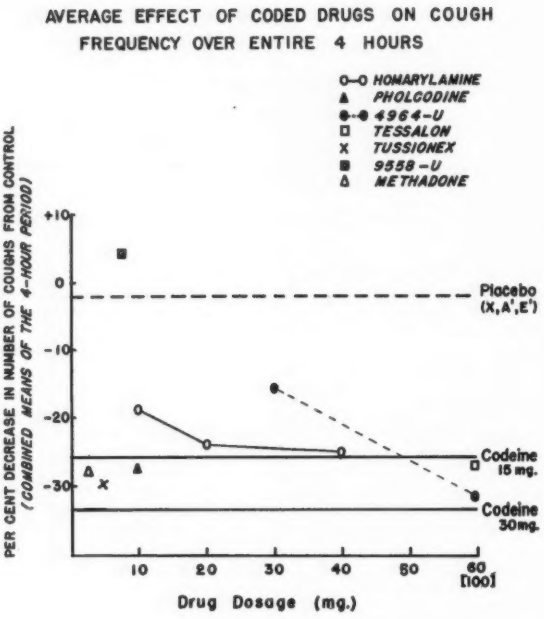

Fig. 1. Antitussive effect of coded drugs compared with placebo and codeine based upon the per cent decrease in cough frequency from the control level over the entire 4 hour test period.

the cough "intensity" factor paralleled the changes in cough frequency. This is illustrated in Table III. The only major discrepancy noted was with homarylamine, 40 mg., where the reduction in intensity was not significant at the first and fourth hours, and with benzonatrate at the third and fourth hour periods.

Comparisons between the various drugs and between different dosage levels of the same drug with respect to antitussive effectiveness over the entire 4 hour period were obtained by graphically plotting the per cent decrease in the number of coughs from the control level for each preparation, using the combined mean of the four hourly periods. The three placebos were grouped to serve as the inactive control while the results obtained with 15 and 30 mg. of codeine in our previous study, representing active reference standards, were superimposed. This is shown in Fig. 1. Only one drug, 9558-U, with a mean per cent change of +5.6, fell within the placebo range. Methadone, 2.5 mg., morphinolinylethylmorphine, 10 mg., and benzonatrate, 100 mg., all appeared equiactive with mean changes of —27.8, —27.1, and —26.6 per cent, respectively. This coincided closely with the mean value of —25.6 per cent for codeine, 15 mg. Dihydrocodeinone resin, 5 mg., with a cough suppression of

Table IV. *Effect of placebo and dihydrocodeinone resin on the frequency of cough elicited by citric acid aerosol over a 7 hour period*

	Drug	*Tests per hour*	*Cough interval*	*Mean % change from control*	*Confidence limits (95%)**
A′	Placebo	35	Control		±21.8
			1 hour	− 1.7	NS
			2 hours	− 4.5	NS
			3 hours	+ 3.4	NS
			4 hours	− 3.4	NS
			5 hours	+ 4.5	NS
			6 hours	− 4.5	NS
			7 hours	− 6.2	NS
B′	Dihydrocodeinone resin	50	Control		±27.8
			1 hour	−43.7	S
			2 hours	−39.9	S
			3 hours	−29.1	S
			4 hours	−40.8	S
			5 hours	−40.8	S
			6 hours	−49.5	S
			7 hours	−54.4	S

*Three-factor analysis of variance (subject-hour-order) expressed as 95 per cent limit.

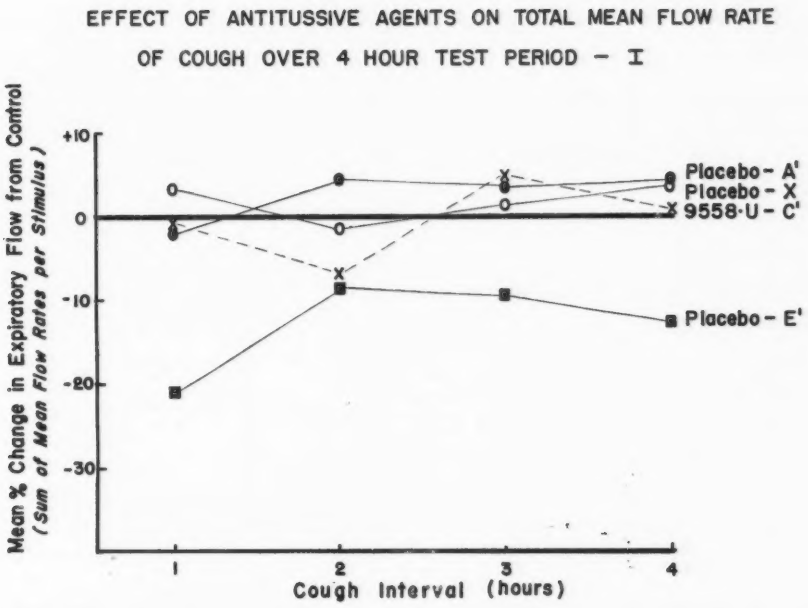

Fig. 2. *Group I.* These drugs showed no significant antitussive effect based upon the per cent changes in intensity factor from the control levels at each hourly period.

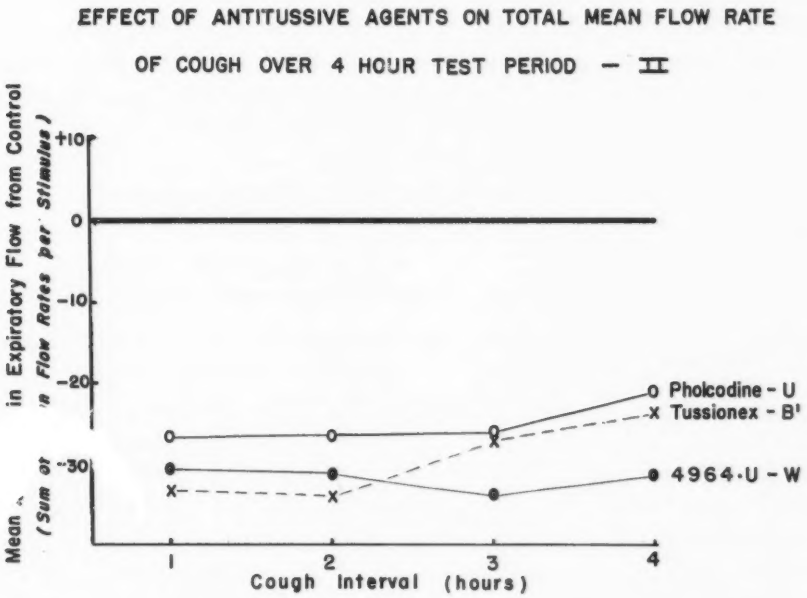

Fig. 3. *Group II.* These drugs exhibited a significant and constant level of cough suppression over the 4 hour test period as evidenced by a fairly flat response curve.

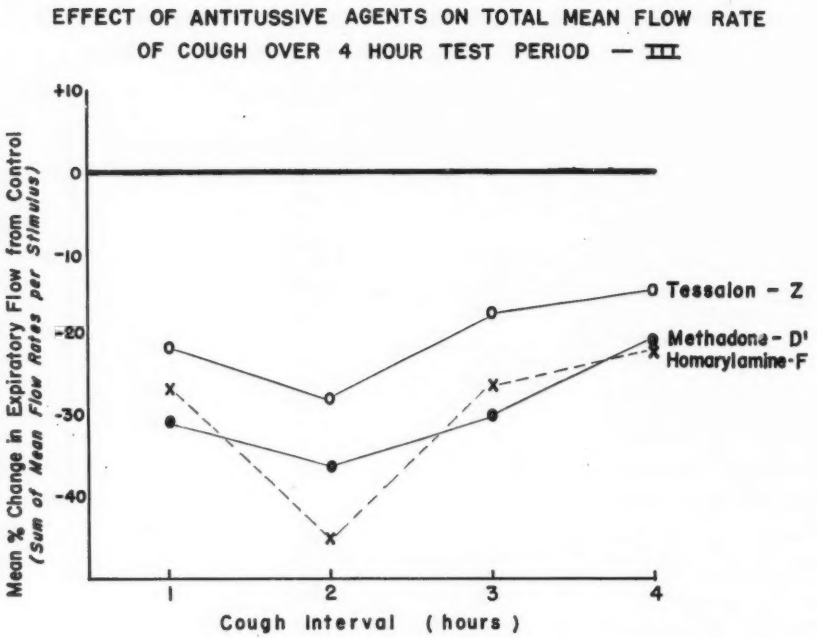

Fig. 4. *Group III.* The drugs of this group exhibited maximal suppression at the second hour with a waning of antitussive activity after the third hour.

—29.5 per cent, was midway between 15 and 30 mg. of codeine. Although a dose-response relationship was observed with both homarylamine and 4964-U, only a slight increase in antitussive effectiveness was noted on doubling of the 20 mg. dose of homarylamine, whereas an increase in effect proportional to the increase in dosage was apparent for 4964-U.

In addition to assessing these agents for over-all effectiveness, it was of interest to compare the hour-by-hour effect of each drug following a single dose. By plotting the per cent change in the product of mean flow rate and volume (intensity factor) for each hourly period, the submitted drugs could be separated into three general categories. The drugs in Group I (Fig. 2), with the exception of E′, exhibited only slight deviations from the "no-change" line at zero. The drugs in Group II (Fig. 3), exhibited a fairly constant level of antitussive activity over the 4 hour test period as evidenced by the flat response curves. Those in Group III (Fig. 4), on the other hand, tended to have their peak effect at the second hour. This is strikingly apparent in the case of homarylamine. Antitussive activity appeared to wane after the third hour. In this respect the agents of Group III paralleled the behavior of codeine.

An illustration of the actual graphic tracings of the cough response of Subject S. I. to a single inhalation of citric acid aerosol is shown in Fig. 5. The differences in response to representative agents from each of the three groups in the frequency of cough and in the intensity factor, obtained by integration of the areas under the pneumotachographic tracings, are evident.

In order to explore further the time relationship, or duration of antitussive activity, two drugs were selected for retesting over a 7 hour period. A′ (placebo) and B′ (dihydrocodeinone resin) were administered to the subjects and tested in identically the same manner as in the preceding study. The results of this 7 hour experiment are illustrated in Tables IV and V. No signifi-

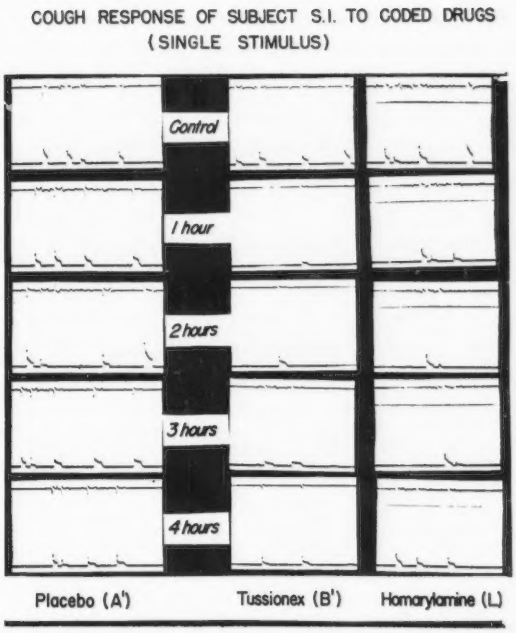

Fig. 5. The effect of representative drugs from each of the three groups on the cough response of Subject S. I. to single inhalations of citric acid aerosol is illustrated in the pneumotachographic tracings.

cant effect was noted with the placebo in either the "frequency or intensity" of cough over the 7 hour test period. Dihydrodeinone resin, on the other hand, maintained significant cough suppression for the duration of the study. It is noteworthy that the results of the first 4 hours of the retest paralleled those of the preceding experiment. This is illustrated in Fig. 6.

Discussion

Since the duration of effective antitussive activity of a drug is as important as the degree of inhibition in the over-all evaluation of cough suppression, it is interesting to speculate on the mechanisms responsible for differences observed between the drugs of Group II and those of Group III. In retrospect, the validity of this observation would have been strengthened by prolonging the test period to the time where all values returned to the control range. From the evidence presented, however, the following possibilities are considered:

1. Differences in the rates of absorption of the various drugs. For example, in a recent clinical study by Cass and Frederik,[4] a sustained release preparation, an ion exchange resin complex of dihydrocodeinone* was found to yield effective antitussive activity over a 10 to 12 hour period.

*Tussionex.

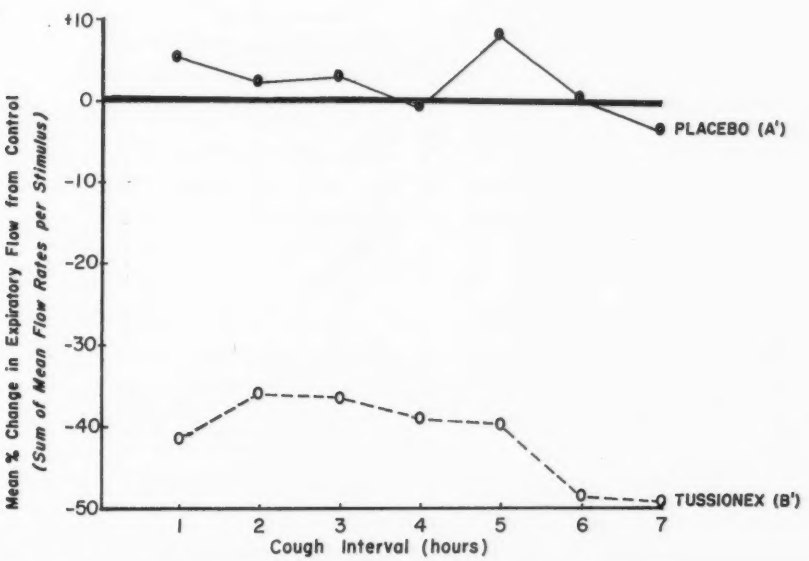

Fig. 6. A comparison of the effect of dihydrocodeinone resin (Tussionex) and placebo extended over a 7 hour period illustrates the persistence of effective cough suppression.

Table V. *Effect of placebo and dihydrocodeinone resin on the intensity (mean flow rate) of cough elicited by citric acid aerosol over a 7 hour period*

	Drug	*Tests per hour*	*Cough interval*	*Mean % change from control*	*Confidence limits (95%)**
A′	Placebo	35	Control		±21.6
			1 hour	+ 5.7	NS
			2 hours	+ 2.1	NS
			3 hours	+ 3.0	NS
			4 hours	− 0.3	NS
			5 hours	+ 8.0	NS
			6 hours	+ 0.3	NS
			7 hours	− 3.6	NS
B′	Dihydrocodeinone resin	50	Control		±18.8
			1 hour	−41.4	S
			2 hours	−36.0	S
			3 hours	−34.4	S
			4 hours	−39.1	S
			5 hours	−39.8	S
			6 hours	−48.9	S
			7 hours	−49.9	S

*Three-factor analysis of variance (subject-hour-order) expressed as 95 per cent limit.

2. Differences in the rates of excretion or degradation. Antitussive activity may be inherent in a metabolite of the administered preparation.

3. Site of action on the reflex arc mediating the cough response. Benzonatrate, a congener of tetracaine, has its principal effect on the stretch receptors of the lungs modifying the response to lung inflation which constitutes the first phase of the act of coughing.

While a double blind study of this type is essential to distinguish between the effective and ineffective agent, the applicability of this technique in antitussive investigation may go beyond the limitations of the double blind study. For example, it would seem that the experimental method could be an excellent tool in exploring the pharmacologic potency of a single effective drug with respect to dose-response and time-response relationships.

In addition to the experimental studies reported, simultaneous long-term clinical trials have been conducted with many of the same coded preparations in patients with chronic lung disease including bronchiectasis, chronic bronchitis, asthma, and pulmonary emphysema. These studies provide additional information such as the incidence of adverse side effects, which could not be evaluated by the experimental method. This will be reported at a later date.

Summary

Experimental cough induced by citric acid aerosols was used to separate the thirteen coded drugs submitted for evaluation into effective and ineffective antitussives. This technique yielded objective data in the form of graphic tracings of the cough response which could be subjected to statistical interpretation.

Differences in both degree and duration of effect on the cough response as measured by changes in the number and "intensity" of the coughs permitted the grouping of these agents into three general categories:

Group I. Insignificant cough suppression exhibited by placebos X, A′, E′, and 9558-U.

Group II. Sustained cough suppression over the 4 hour period represented by mor-

phinolinylethylmorphine, dihydrocodeinone resin, and 4964-U, 60 mg.

Group III. Peak antitussive activity between the first and third hours with waning of effect at the fourth hour, illustrated by benzonatrate, methadone, and homarylamine, 20 mg. Results of earlier studies indicated that codeine, 15 mg., behaved in a similar fashion.

A repeat study employing dihydrocodeinone resin and placebo A′ revealed significant antitussive activity of dihydrocodeinone resin over a 7 hour period as compared with placebo.

In the over-all assessment of antitussive activity, employing the combined means of the decrease in "frequency" of cough over the 4 hour test period, methadone, morphinolinylethylmorphine, benzonatrate, and homarylamine, 20 and 40 mg., appeared equal in action to codeine, 15 mg. Dihydrocodeinone resin and 4964-U, 60 mg., exhibited an effect between that of 15 and of 30 mg. of codeine.

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Developing and testing of new drugs by the pharmaceutical industry

The developing and testing of new drugs is an important responsibility of the pharmaceutical industry. The first tests are "screening" procedures that are intended to uncover leads indicating possible therapeutic usefulness. After thorough pharmacologic and toxicity testing in experimental animals, a new drug is ready for the initial trial in a human subject.

It is practical for a pharmaceutical manufacturer to maintain a clinical research unit and a staff of investigators for performance of the initial human trials. A new therapeutic agent is then placed in the hands of a limited number of experienced and qualified investigators so that adequately controlled studies can be conducted under close observation. In some instances, as a final evaluation, the drug is subjected to a broad clinical trial by a large number of physicians. If properly conducted, important information can be obtained by the use of a drug under conditions found in the practice of medicine. Evidence of hypersensitivity cannot be detected by limited trial. The clinical investigator must plan the study with great attention to detail. Every effort must be made to avoid bias.

In the development of the drug the work of the pharmaceutical chemist is important in preparing a satisfactory dosage form; the control division must establish criteria for the composition of the ingredients as well as for the final product and must prove the stability. Publication of reports in the scientific journals must include methods and controls for clinical studies and the results. A group of investigators can use one experimental design and pool the findings in a single paper. The investigator should report all data to the manufacturer as quickly as possible.

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The development and testing of new drugs are important responsibilities of the pharmaceutical industry. The physician expects a new drug to be thoroughly studied before he is asked to use it in his practice. The "testimonial" type of clinical report is no longer acceptable. Information based on adequately controlled studies is demanded before the claims made by the pharmaceutical manufacturer are accepted. Dana Atchley[1] called attention to the change in the attitude of the physician which has occurred during the first half of the twentieth century. The doctor is no longer an "empiric follower" of tradition. He is trained in laboratory science as well as in clinical medi-

cine. Early in his career he may acquire a modest critical ability to interpret scientific data.

The manufacturer must also have facts regarding the advantages and limitations of a new drug before he can commit the large sums of money that are needed for large-scale production. Data to fulfill the requirements of the Food and Drug Administration must be collected so that permission for interstate marketing can be obtained. Thus, the evaluation of new drugs has come to occupy a major place in pharmaceutical manufacturing. It requires the expenditure of a large amount of money and the full-time efforts of many persons.

Screening

The testing begins with "screening procedures." In our never-ending search for new therapeutic agents a very large number of substances must be examined for properties that might possibly give them a place in the diagnosis and treatment of disease. These materials come from many sources—the organic chemist synthesizes thousands of compounds, and from plant or animal tissue extracts come a wide variety of substances that must be subjected to the screening tests. In recent times a veritable flood of broths from growths of microorganisms from soil has been made available for examination. All of these will be subjected to a series of screening tests intended to detect therapeutic activity in any number of fields. These preliminary tests cover a wide range of procedures. Sometimes the first screening can be conducted in a test tube—for example, the testing of crude antibiotic for its ability to inhibit growth of organisms. Recently tissue culture has given the biologist an opportunity to screen new drugs by noting their effect on living isolated cells. This has proved to be very valuable in the search for substances that will halt the growth of tumor cells or that will be active against viruses.

The administration of the test substance to an intact experimental animal, however, remains the most common form of "screening." Sometimes an abnormal state is created in the animal—for instance, by removal of the endocrine gland or by constriction of the renal artery to produce sustained hypertension. Sometimes, as in testing substances that might affect behavior, very elaborate equipment is employed for continuously recording the activity of the animal. In some instances a potential drug is tested for its effect on physiologic functions, such as excretion of urine or cardiac output. Special tests are devised for measuring the effect of a drug, i.e., production of a standard pain stimulus in order to measure the analgetic activity. These are but a few of the screening procedures employed in the pharmacology laboratories in the quest for new products. At best, all that is expected from them is a suggestion or a "lead." Whenever evidence of a possible therapeutic use is elicited, extensive investigation of the pharmacologic properties is conducted.

Toxicity studies

Determination of acute toxicity is a prerequisite to making tests in experimental animals. This is expressed as LD_{50} (lethal dose for 50 per cent of animals), and from initial trial in animals an ED_{50} (effective dose for 50 per cent of animals) can be derived. The difference between these values provides the first indication of what the "margin of safety" or "therapeutic ratio" is. When it is small, difficulty in the therapeutic use of the substance in man can be anticipated. A large margin of safety is a desirable property in a new drug.

When the more elaborate and detailed studies of the effect of the new drug have confirmed the results of a screening procedure, continued administration of the drug to several species of animals is begun as a test for toxicity. Tests for organ function are conducted in these animals. Adverse effects on renal, hepatic, or bone marrow function may be the reason for halting further development. The rate of growth of young animals is an important criterion of toxicity. Any decrease in the expected rate

of growth would be considered undesirable.

Testing for chronic toxicity has become a well-organized and, at times, a complicated procedure. The pharmaceutical manufacturer must maintain many facilities and a competent staff to conduct the long-term studies that are required. Just to be sure that every animal receives the proper quantity of the drug each day is not a simple task. These animals should receive a daily dose much larger than was needed to produce the pharmacologic effect.

It is customary at the end of 30 days to sacrifice some of the animals that have been receiving the drug. Although they may have exhibited no apparent deleterious effect, their tissues are subjected to careful microscopic examination by a trained pathologist. This individual must have knowledge of abnormalities that occur in animals that are unrelated to the effect of drugs. The remaining animals continue to receive the drug, and if a decision to begin clinical evaluation is made the toxicity studies are expanded.

There are no "short cuts" in preparing for clinical trial. Today it is generally accepted that no drug should be given to a human until it has been administered at least 30 days to experimental animals and the toxicologic tests are complete.

Clinical application

Within our Company, the decision to launch a clinical program is made by a conference of representatives from the chemistry and pharmacology or biology divisions with the physician from the clinical research section. Sometimes this is an easy decision, but in other instances, when many factors must be considered, it may be necessary to repeat or extend the studies in experimental animals.

The evaluation of a new drug in humans can be divided into three phases. The first begins with the administration of a single dose and continues until the amount that will produce the desired therapeutic effect or an undesirable side effect is established. The drug is then given in repeated doses to a small number of patients to establish acceptability.

The second phase is an extension of the first. The number of clinical investigators is increased. During this period carefully controlled studies by experienced investigators are initiated in order to confirm the therapeutic effectiveness and make comparisons with existing drugs. Special techniques which are intended to demonstrate the mode of action are often employed. These investigations provide the basic data on which the final decision is made to proceed with the development of a marketed product.

The final stage is the broad clinical trial. Obviously there is less ability to control conditions during this period of study than during the first and second stages. The purpose of this phase of the clinical trial is to study the drug as it will eventually be used by practicing physicians.

Clinical trial programs vary a great deal. For instance, the procedure for evaluation of a drug to be used for the treatment of a rare disease is different from that followed to test the effectiveness and safety of a compound to be used broadly, such as an analgesic. Also, if the drug is to replace a safe therapeutic agent, the clinical trial may differ from the method employed when the drug is intended for treatment of a heretofore fatal disease.

Another factor that may determine the extent of the clinical trial and its rate of expansion is the ability of the laboratory to supply the compound. Methods for large-scale production may remain to be developed, and often it is not feasible to undertake this until proof of the drug's clinical usefulness and acceptance by the physician has been obtained.

Initial clinical trial. The usual procedure for initiating a clinical study has been to present the results of observations in experimental animals to an investigator who is associated with a research laboratory in a medical school or large center. This has many disadvantages, and is becoming less attractive to competent clinical investi-

gators. As we have shown,* it is possible for a pharmaceutical manufacturer to develop a clinical research unit in a medical center, staffed by full-time employees. The physician responsible for the initial trial must be experienced in clinical research, and he must have an adequate staff and laboratories so that the first administration can be made with minimal risk. He would serve in the outpatient clinics or on the regular wards when not engaged in the evaluation of a new compound. This maintains physician-patient relationship and gives the investigator continuing experience in clinical medicine. It also provides an opportunity to become acquainted with a large number of people who might be willing to volunteer for participation in a clinical research project. Our own experience has proved this to be a practical and efficient procedure for the initial clinical studies of many new therapeutic agents. Another advantage to industry is that the physician-investigator employed by the pharmaceutical manufacturer may be able to evaluate several similar compounds before one is picked for further study.

The selection of a dose for the first administration of a new drug to a human subject is difficult. There is no formula for extrapolation from animal dose that which is safe for human subjects. A cardinal principle is to select the minimum amount expected to produce some therapeutic effect, on a weight basis, from results in dogs and/or monkeys, and then give only a portion of this amount as the first dose.

Sometimes normal subjects are employed as volunteers for the initial trial. They should be under constant observation by a trained investigator until all possible effects of a single dose have disappeared. In some instances the first use of the drug must be in actual treatment of disease. For example, the first use of a new antibiotic would be in the treatment of an infection which is not severe or life threatening and one caused by organisms that are known to be susceptible to the new drug. Treatment with the new drug might also be undertaken when resistance to existing antibiotics was present.

After experience with a single dose has been gained from several subjects, the amount given is increased by small increments, one patient at a time. This, a slow and tedious process, must be continued until the therapeutic result occurs, a toxic symptom is detected, or the maximum practical dose (an amount which might be placed in a capsule or tablet—usually not more than 1 Gm.) has been given and neither therapeutic nor toxic manifestations occur. As has already been described, in many instances the preliminary clinical investigation can be made very satisfactorily in a clinical research unit maintained by the manufacturer of the drug.

Second phase of the clinical program. Having demonstrated a therapeutic effect in a small number of patients, a representative of the clinical research section of the pharmaceutical manufacturer, preferably the same individual who conducted the initial trial, will present the results to several experienced investigators. It is also important that these individuals be expert clinicians who are recognized for their knowledge of the disease to be studied. They must be familiar with the natural history of the disorder that will be investigated. Their studies will be intended to confirm and extend the observations made during the initial trial.

At this stage it is often possible to conduct clinical-pharmacologic tests. An example of this type of procedure is our experience with an antitussive agent. When evidence of its therapeutic effect had been obtained in a few subjects and there was some indication of the amount needed as single dose, Dr. Hylan Bickerman was asked to test the drug for its ability to control cough induced by inhalation of citric acid. He had developed a device for recording cough response in normal subjects and

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alterations in the cough pattern produced by therapeutic agents.[2] He was able to compare the new drug with codeine. This comparison would have been difficult, if not impossible, if it had been based on observations of the therapeutic effect in patients with a cough associated with pulmonary disease. From the results of this study it will be possible to plan further clinical trials.

The need to avoid bias in making comparisons of therapeutic effectiveness of a new drug with an existing substance in general use has increased the interest in the "double blind technique"—a procedure in which neither the subject nor the observer knows whether a placebo, a control preparation, or a new drug is being given. Unfortunately, as Modell and Houde[9] have noted, it has come to be looked upon as a "cure all" for all problems in clinical research. As these authors have emphasized, however, the use of a placebo does not validate an otherwise poorly designed experiment.

In the attempt to compare a new drug with a blank and a known preparation, consideration must be given to the order of administration. The effect of a compound may continue after its administration has stopped; the course of the disease may change during the period of study; the intensity of pain may change. These are but a few examples of the reasons why it is important to randomize the order of treatment when more than two drugs are involved in a therapeutic trial. One method for accomplishing a "planned randomization" is the use of the Latin square.[4] This mathematical device was first described by Leonhard Euler in the eighteenth century. It is called a Latin square after Euler's practice of labeling each cell in the square with a letter. It was first employed in problems relating to agriculture, but has come to be a useful tool in clinical research. In Fig. 1 is an example of a 4 by 4 Latin square. It is so constructed that each letter appears once in every horizontal line and in each vertical column. This design provides an order for administration of four medicaments to four subjects or four groups of patients. A new drug "A," a blank "B," an active control (a known drug) "C," and a larger dose of A, would be given for four periods. This might be one day to one year. At the end of the study every drug would have preceded and followed every other drug one time. The Latin square is particularly valuable when the criterion for a therapeutic trial is a subjective response.

	TIME			
PATIENTS	PERIOD 1	PERIOD 2	PERIOD 3	PERIOD 4
GROUP 1	A	B	C	D
GROUP 2	B	D	A	C
GROUP 3	C	A	D	B
GROUP 4	D	C	B	A

Fig. 1. Four by four Latin square. For comparing four test substances in four patients or four groups of subjects. Each letter follows and precedes every other letter one time.

When only two drugs are being compared, or when the investigator wishes to demonstrate the difference between a blank and the new drug, a "cross-over" design should be used. In this type of trial the subject serves as his own control.

Allocation of patients is also very important. When one group will serve as controls and another will receive the new drug, it is essential to randomize the distribution. This can be done by a table of random numbers or by chance determined by flipping a coin.

Occasionally the double blind technique is employed when objective responses are being measured. For example, in the investigation of the effect of beta sitosterol on serum cholesterol by Shipley,[11] an attempt was made to eliminate any possibility

that merely taking medicine might motivate the patient to observe the prescribed diet, by giving a placebo pre- and post-treatment.

Another important purpose of the second stage of clinical evaluation of a new drug is to obtain evidence of its safety. A battery of laboratory tests is performed prior to the first dose of the drug, and they are repeated at regular intervals over a period of months. Examinations for liver and kidney function are essential, and a careful check on hematopoiesis is also conducted. It is important that the tests be performed by competent technicians. An error in these examinations could cause toxicity to be missed, or an unwarranted stigma might be placed against the drug. The latter could cause cessation of clinical studies and the loss of a valuable therapeutic agent.

When results of laboratory tests suggest some toxicity, further development of the drug will depend upon the nature of the disease for which the drug was intended. Some toxicity is allowable if the drug offers treatment for a heretofore fatal illness. If it only provides a substitute for a safe, existing therapeutic agent, however, the new compound must pass rigid tests for both safety and effectiveness.

Third phase, broad clinical trial. The need and extent of this part of the clinical trial will vary according to the nature of the drug. Although it will be less well controlled than the preceding studies, a great deal of valuable information can be obtained if it is properly planned and executed. It should not be considered as a "promotional gimmick." The incidence of side effects and the occurrence of reactions due to hypersensitivity cannot be determined until a larger number of patients have received a drug. Our recent experience with Carbutamide, a hypoglycemic agent for oral use in the treatment of diabetes, is an example of the value of a broad clinical trial. In this instance the results of carefully controlled studies in the management of 600 patients had been reported before an extensive trial was initiated. No serious untoward symptoms had been observed. After the administration of several thousand doses of Carbutamide, however, the occurrence of undesirable effects became apparent. The clinical trial was halted and further development was discontinued.

When a broad trial is undertaken it is important that the physicians be supplied with all of the pertinent information about the drug. When this stage has been reached, a definite dosage schedule has been established. It is essential that the physician be given instructions regarding the selection of suitable patients. Usually it must be emphasized that adherence to the dosage recommendations is imperative. The physician should also be given a questionnaire so that he will know in advance the information that is desired. Placebo and the double blind technique are not often necessary in this phase of clinical trial.

Reducing bias in clinical research

The term *bias* is derived from the word *biais,* which means a slope or slant. In a clinical trial program it is of utmost importance that all chances for bias, either favorable or unfavorable, be eliminated or at least reduced as much as possible. Bias may occur in several forms. First, the investigator himself is generally recognized as the source of bias. His enthusiasm or antagonism may have a positive or negative effect. As Shapiro[10] has pointed out, the attitude and actions of the physician may influence the response of the patient to a new drug.

Recently Kast and Loesch[8] conducted an experiment which illustrated the interaction between patient and medical environment. In this study consistent and deliberate variation in the physician's attitude was introduced. Twenty patients suffering from an anxiety syndrome with primary gastrointestinal complaints were observed during a period in which the expected benefit of a combination of tranquilizer and anticholinergic drugs was stressed. Two physicians

saw the patients at weekly intervals. They were careful to make favorable comments regarding progress in the presence of each patient. They were always on time; the patients were seen promptly, and everything was done to make each visit a pleasant one. During the second period the physicians continued to be solicitous and kind to the patient, but a placebo was substituted for the active drug. In the third period the patients received the same drugs, but in a capsule rather than in tablet form. They were told it was different medication. The physicians were changed and this time only one saw the patient. He was deliberately antagonistic. He was purposely late and never offered a favorable comment. During the first period 18 of 20 patients reported marked improvement. When the placebo was given, only 3 patients maintained their status. In all others complaints recurred. During the third period, of the group that had benefited during the first stage, 3 did not experience relief from anxiety and gastrointestinal complaints became worse in 5. This provides a very definite example of how the physician's behavior toward the patient can influence and alter results of a clinical trial.

Another source of bias may be the patient. Wolf[13] has called attention to the hazard of using persons who refuse treatment as control subjects. In addition, normal persons who volunteer for clinical trial may represent a distinct personality pattern. Patients, in their desire to please their physician, may introduce bias. An example of cooperation by the patients and "salesmanship" on the part of the investigator is illustrated by the experiment conducted by Gruber.[5] Eleven patients on a research ward were told that the purpose of an experiment was to change their sleep pattern. On alternate nights they were given a red capsule and were told it would make them sleep. On the next night they were given a white tablet and told it prevented sleep. Both preparations were placebos. This procedure was continued for 10 consecutive nights. Each night the patients were reminded of the result to be expected. Next morning they were interviewed. Three subjects were very cooperative. They slept or stayed awake just as they were told. Three patients who may have consciously or subconsciously resented being "guinea pigs" responded in exactly the opposite manner from that requested.

Dr. Gruber has also demonstrated that merely increasing the dose of a placebo can influence the occurrence of side effects. A group of patients were each given one placebo capsule. Several reacted by describing side effects. And when those who had not experienced any untoward symptoms were given two capsules of the same placebo, several complained of headache, dizziness, etc. Dr. Gruber has devised a method for avoiding this source of bias when the dosage of a new drug is being determined. He uses three placebo tablets as an initial dose. This permits three increments in the amount given at one time, without the subject being aware of a change. The same result could be obtained by using capsules of the same size and appearance, but containing varying amounts of the drug to be tested.

Another source of bias can be the attitude of paramedical personnel who may talk with the patient. Enthusiasm or antagonism of a nurse toward an experiment can influence the attitude of the patient. In fact, the inflection in the voice of the observer or the way the question is worded can be a factor in studies involving subjective response.

Dr. Gruber has employed a pegboard when evaluating an analgesic. Questions are printed on the board and beside each are holes, one for the affirmative and another for a negative reply. The nurse hands the board to the patient who is asked to place pegs in the hole after each question. It is our opinion that this simple procedure reinforces the double blind technique. Although neither the nurse nor the patient knows when an active drug or placebo is being given, nevertheless, inadvertently—merely by the phrasing of the question—a

source of bias could be introduced which could influence the response.

Another problem in any clinical trial arises when some of the patients do not complete the series of test drug, control, and placebo. No completely satisfactory adjustment can be made for such missing data. If these subjects are excluded and not reported, the frequency of side effects as well as the number of failures or even successes may be altered. The usual reason for a person's stopping treatment is occurrence of side effects or failure to obtain benefit. Thus it is likely that the greatest number of poor responses would be found in the group that did not complete the study. The investigator should make note of this in his published report. At least, mention should be made that there were subjects who did not complete the study.

In our effort to avoid bias, we must guard against making the study so complex that sensitivity of the method is lost. Modell and Houde[9] have pointed out that a negative conclusion is meaningless unless a control is incorporated which demonstrates a positive result.

Development in areas other than testing

The preparation of new drugs for clinical study occupies an important place in pharmaceutical manufacturing. Sometimes it is a very simple task of merely filling capsules or making tablets. Here, as in clinical trial, unless attention is paid to details, factors can be introduced that will negate all care in execution of a clinical study. For example, stability of the drug must be determined, and it must be established for the form in which the drug will be given. Disintegration of the tablet may be an important factor. The pharmacist working in the pilot plants must be kept informed of any clinical experience so that he may work to improve the pharmaceutical form.

It is very unsatisfactory to depend on patients for information as to preference for taste of a liquid preparation. It has been found that a taste panel of volunteers, if properly selected and using double blind technique, can give very satisfactory information as to taste preference.

As the clinical trial progresses, there are many important tasks that must be completed prior to submission of the new drug to the Food and Drug Administration. Methods and tests for control of composition of ingredients as well as standards for the final product must be developed. The package must be designed, and in some instances, as with aerosols, this can be a very important part of the clinical trial. All of these efforts must be coordinated, and this requires communication between all groups.

The selection of a suitable name may present many problems. It is no longer an easy task to find one that is not already in use.

Recording and publishing results

As in other types of experimentation, attention must be paid to the recording of results in the course of a therapeutic trial. The design of the forms or questionnaires to be used by the observers is an important part of the planning of a study for the evaluation of a new drug. When the results can be measured objectively, keeping the data is relatively easy, but when a subjective response is the basis for the assessment of the therapeutic effect, the problem of recording data can be difficult. This must be done without introducing bias.

Usually the investigator interviews the patient and records his or her description of the effects of the drug. This is often done by grading the response from 1 to 4. Since the individual who is being treated outside of the hospital may be seen only at weekly intervals, there is a chance the patient may fail to recall the events that followed the ingestion of the drug. Some investigators have found that the use of a daily report card by the patient is helpful in getting information about the subjective response. Instances have been known in which the subject completed all reports for a week while waiting to see the physician.

We have used a daily telephone call[3] as a practical method of securing information.

If all precautions to avoid increasing bias are observed, and if a time for the call is fixed, this is a satisfactory method for geting a report from the patient each day.

After data have been compiled they must be collated, interpreted, and published. If the study has been planned properly and executed carefully, the results can be subjected to mathematical tests for significance. Statistical analysis, however, is not a remedy for "sloppy" measurements. As we have mentioned, the study is meaningless unless sensitivity of the method has been demonstrated. Sometimes the importance of the observation made is lost in unnecessarily complicated mathematical terminology. A result may be statistically significant but not have therapeutic importance. Contrariwise, valid clinical observations may be lost in a maze of statistical detail. A knowledge of the natural history of the disease as well as the investigator's own experience in clinical medicine must be applied in interpreting the results.

Prompt and complete reporting of the information obtained from a therapeutic trial to the pharmaceutical manufacturer is an important responsibility of the investigator. The results should also be published in a scientific journal. The design of the experiment and the method for allocating patients to the treatment or control group must be included. The measurements must be described and it should be clearly stated whether the results were obtained by "blind" technique or if the observer was aware of the treatment being given. As Waife[12] has pointed out: "These are the 'special research methods' in clinical trials and require the same meticulous and detailed presentation as do, for example, the descriptions of laboratory techniques in biochemical journals."

One procedure for reducing the number of published reports is to plan an experiment in which several investigators, working in different institutions, follow a prearranged experimental design. All of the results can be combined in a single publication. A conference of investigators before and after the study is essential. It is very important that all participants share in formulating and interpreting the results. This procedure was used by Gruber[6] in a clinical evaluation of propoxyphene. In this instance groups of investigators administered codeine phosphate, propoxyphene hydrochloride, and placebo to patients having chronic pain of moderate severity. The same experimental design was employed in seven institutions in different cities in different kinds of hospitals. One hundred and one patients were observed during the administration of five medications. (Two doses of the analgesics were used.) Randomization was planned and each medicament was given for a period of 3 days. All observers followed the double blind technique. Medication was supplied in identical-appearing capsules in a numbered envelope. At each institution the code was placed in a sealed envelope so that if any reason for termination of the therapeutic trial should arise, the physician could know at once the drugs the patient had received. At the end of the study it was possible to have an analysis of variance of 1,515 daily pain scores obtained from 101 patients. The combined results were published in *The Journal of the American Medical Association* before the new analgesic was made available as a marketed item. This report provided the practicing physician with well-controlled evidence to support the claim that, on a weight basis, propoxyphene hydrochloride and codeine phosphate are equally effective in reducing discomfort of chronically ill patients.

It is our opinion that cooperative experiments merit development by the pharmaceutical industry. The procedure would not be applicable to all types of clinical research, but it does offer a means for accurate reporting of a therapeutic trial without scattering the results in several journals. If the study has been designed properly, negative results should have significance and therefore would deserve to be reported. Although the therapeutic efficacy may not have been sufficient to justify complete development of a new drug, nevertheless the

fact that a compound or preparation failed to produce results or caused undesirable side effects is important information for other investigators. A brief note regarding negative results should be published, so that it will be catalogued in the medical literature. It is very difficult to accept the manufacturer's statements regarding therapeutic effectiveness when the only source of information is personal communication between the manufacturer and the investigator. Acceptance of a new drug should not be expected unless the physician is given an opportunity to read the complete report of studies including the experimental design on which the manufacturer bases his claims.

Conclusions

The development and testing of a new drug is a long and arduous process. The responsibility of the manufacturer for the clinical evaluation of each new drug is continuously increasing. This has necessitated the expansion of facilities and the number of employees. In 1958, at Eli Lilly and Company, approximately 1,300 persons of a total of 8,000 employees in the United States were devoting their full time to research, development, or control. All this effort is destined to give the practicing physician the new therapeutic agents that will enable him to provide the best medical care. Proper use of these additions to his therapeutic armamentarium requires that the physician have complete information about their properties, so that each drug may be used with maximum safety for the patient and in a manner that will give optimum results.

In his opening remarks at a symposium in London (1958) on quantitative methods in human pharmacology and therapeutics, Sir Charles Harington[7] called attention to the possibility that, in our effort to assess therapeutic effect, the keen and accurate observations needed will on occasion yield new insight into the disease process being studied, and thus advance medicine in the general sense. The pharmaceutical industry has come to occupy an important place on the "health team."

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Proteolytic enzymes: a therapeutic evaluation

A promising development of recent years has been the application of proteolytic enzymes to the therapy of disease in the human. Originally applied directly to the diseased area to effect a local enzymatic débridement, proteolytic enzyme therapy is now being extended on a systemic basis to widen considerably the scope of its applicability. This report summarizes the current state of knowledge of proteolytic enzymes as therapeutic agents. It indicates those areas where the usefulness of proteolytic enzyme therapy is well established and those areas where the efficacy of such enzyme therapy is less well accepted or under active clinical investigation. Though much has been accomplished already at the clinical level, particular stress is placed on the problems which remain to be solved if proper exploitation of this new and exciting area of pharmacology is to be achieved.

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The recognition of the importance of enzymes in biologic phenomena has been a prominent feature of the current scientific surge. Application of this information to medicine has resulted in significant contributions to the understanding of disease and the development of clinically useful diagnostic aids. Another logical application has been the use of enzymes as therapeutic agents, a development which has grown rapidly in recent years. This report briefly reviews and evaluates the current status of the therapeutic use of proteolytic enzymes in human disease. However, since much of this area of pharmacology remains in a confused state, the major purpose of the report will be to re-emphasize the potential usefulness of proteolytic enzymes as therapeutic agents in certain disease states and, more importantly, to define the developments required to exploit properly this relatively new and promising field of therapeutics.

In general, proteolytic enzymes have been used therapeutically in four areas: (1) as oral agents for specific gastrointestinal disorders; (2) as local agents to débride or solubilize collections of proteinaceous material which either cause or foster disease; (3) as anti-inflammatory agents; and (4) as thrombolytic agents in the treatment of thromboembolic disorders.

I. The use of proteolytic enzymes as oral agents for gastrointestinal disorders

Proteolytic enzymes have been used orally as anthelmintics, in the treatment of chronic pancreatitis, and in the conserva-

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tive management of an obstructing bolus of meat in the esophagus. Orally ingested proteolytic enzymes in common use apparently do not affect normal mucosal cells nor are they significantly absorbed through the intestinal mucosa.

Ficin, the powerful proteolytic enzyme obtained from the sap of the *Ficus laurifolia*, a South American fig tree, has been used by the natives for centuries as an anthelmintic. Though it will not digest the healthy mucosa of the gastrointestinal tract, ficin will digest a number of species of living worms including Trichocephalus, Oxyuris, hookworm, Ascaris, and Strongyloides. However, ficin's therapeutic usefulness is limited since, in the presence of intestinal irritation, it will act upon the mucous membranes, producing erosions and hemorrhage. As more potent and less toxic anthelmintic preparations have been developed, standard pharmacology texts[1] no longer list this agent.

Preparations containing either pancreatic enzymes or papain have served as useful agents in the management of the more serious nutritional problems seen in association with chronic pancreatitis.[2,3,4] Since these enzyme preparations are used for replacement therapy, whole pancreatic enzyme extracts have enjoyed the greatest popularity. The majority of patients with chronic pancreatitis can be maintained without supplementary oral enzyme therapy; when they require it, large amounts are necessary for a salutary effect.

The oral use of proteolytic enzymes for rapidly softening and digesting a bolus of poorly masticated meat which has acutely obstructed the esophagus is often attended by a dramatic effect.[5,6] Papain preparations have enjoyed considerable commercial success as meat tenderizers and appear to be more efficacious than trypsin or pancreatic enzymes, for they have the ability to act over a wide pH range. In selected cases, the institution of this conservative enzymatic procedure is frequently gratifying and worthy of trial; additional diagnostic procedures should be instituted to uncover an underlying pathologic process. The successful medical management of a persimmon phytobezoar with papain and sodium bicarbonate has been reported recently.[7]

II. The local use of proteolytic enzymes as débriding agents

Basis of their use and general considerations. The concept of using proteolytic enzymes to digest dead tissue as an adjunct to the management of dirty, infected wounds is an old one and probably stems from the observations of the natives of tropical countries where the papain-rich latex obtained by scratching the skin of the green fruit of the papaw tree *(Carica papaya)* has long been used for the treatment of eczema, warts, ulcers, and other types of foul sores. Before the turn of the twentieth century, articles appeared on the use of papaya latex preparations for treating sloughing ulcers, removing impacted cerumen, and dissolving diphtheritic membranes,[8,9,10] and on the use of pancreatic enzyme preparations for dissolving the "tough lining membrane of fibrinous lymph, and the loose coagula and the sloughing diseased tissue" of tuberculous abscess cavities.[11]

Despite occasional flurries of interest in the use of enzymes during the succeeding years, the modern era of local enzyme therapy had its origin in the period immediately following World War II; its development stems from two entirely unrelated areas of investigation: the nature of the spreading factor in tissues, and a study of the enzymes elaborated by hemolytic streptococci. The introduction of hyaluronidases[12] to enhance absorption from the connective tissues and of fibrinolytic and nucleolytic enzymes as local enzymatic débriding agents[13,14] can be attributed in each case to a well-founded developmental approach utilizing basic knowledge and employing appropriate quantitative and qualitative techniques to define the objectives of such therapy, to elaborate the principles involved, and to demonstrate the effects. The latter studies,[13,15-20] which are more relevant to this report, demonstrated that

the major insoluble constituents of inflammatory exudates, fibrin and the desoxyribonucleoprotein derived from the nuclei of dead degenerating cells, could be rapidly lysed by the local application of a mixture of enzymes obtained from the secretory products of certain strains of hemolytic streptococci. The major constituents of this enzyme mixture, streptokinase (an activator of plasminogen, the naturally occurring precursor of a proteolytic and fibrinolytic enzyme of human plasma) and streptodornase (streptococcal desoxyribonuclease) provided the basis for an enzymatic débridement. When properly applied to selected patients they proved capable of cleansing infected surfaces of their inflammatory exudate but without harm to living tissues; facilitating the drainage of areas of loculated purulent, sanguineous, and fibrinous accumulations; promoting the liberation of hidden bacteria, thus enhancing their exposure to antimicrobial agents and native immune forces; and increasing the rate of repair of previously infected wounds.[14,21-26] These well-documented observations served as a framework for a rational approach to the local use of proteolytic and nucleolytic enzymes in the management of disease. Many investigators working not only with streptokinase-streptodornase mixtures but with other proteolytic and nucleolytic enzymes, as well, have rapidly extended the concept of an enzymatic débridement for practical use in treating diverse surface infections, chronic draining sinus tracts, and accumulations of clotted blood and inflammatory exudate arising within various body cavities. In a number of instances the effectiveness of such enzyme therapy has been dramatic; in general, its use has allowed for more conservative management of many disease situations which formerly required extensive surgical intervention and prolonged periods of incapacitation. Abundant studies, whose reveiw is beyond the purpose of this report, attest the usefulness of this type of débriding procedure in the treatment of various diseases; the reader may review this aspect of the subject by consulting selected references.[27-32] Since this form of therapy is no longer novel, little purpose will be served by further extolling its merit. Rather, it would be more appropriate, in terms of necessary future developments, to give serious consideration to the factors which have limited the extent and scope of its practical application. These limiting factors have included:

1. Substrate considerations. A number of pathologic substrates (burned skin, dead bone, necrotic connective tissue) are not readily digested by the enzyme preparations currently available for clinical use.

2. Presence of underlying disease. Frequently enzyamtic débridement is instituted for disease situations which are the result of more complex disease problems. Unless it is recognized that, under many circumstances, local enzyme therapy is only an adjunct to the management of a problem for which additional therapeutic measures are also necessary, the results may often be disappointing.

3. Undesirable side effects. Most of the currently available preparations are locally irritating and also may produce pyrogenic reactions when placed within closed body cavities.

4. Practical technical difficulties. Adequate enzyme therapy requires that the enzymes be brought into direct contact with the affected tissues, maintained there in adequate concentration for a sufficient time to produce the desired effect, and followed by appropriate and adequate drainage. The practicing physician, in order to simplify the clinical use of enzymes, often has overlooked these principles, and, as a consequence, has found it difficult to reproduce the results achieved by the careful investigator.

5. Economic considerations. Purified enzyme preparations are expensive; when frequently repeated administrations are required, economic considerations limit their use.

6. Enzyme considerations. Despite the mass of clinical observations, there has been

a patent lack of associated correlative laboratory study. As a consequence, the indications for the use of a specific enzyme preparation, or the advantages of one, as compared to another, never have been defined clearly.

Specific enzyme preparations in current use for local débriding purposes.

1. Streptokinase-streptodornase mixtures. The available preparation* is only partially purified and contains a number of other streptococcal enzymes such as a ribonuclease, hyaluronidase, nucleotidase, and nucleosidase, all of which may contribute to the effects observed. The enzyme mixture is essentially free of streptolysin and streptococcal proteinase. Since it does not contain any proteolytic enzymes in the conventional sense, it differs in type from the other preparations to be discussed. In addition, it is the only preparation containing enzymes which act upon nonprotein substrates; much of its virtue lies in its content of streptodornase which rapidly reduces the viscosity of thick purulent exudates.

A unit of streptokinase is defined as that amount which under standard conditions activates sufficient human plasminogen to effect the lysis of a fibrin clot in 10 minutes. It is still unclear whether streptokinase requires another humoral factor (proactivator) to activate human plasminogen.[33] Plasmin, the proteolytic enzyme formed from the latter precursor, is active at neutral pH and, though distinct from trypsin, resembles it in many respects (pH optima, types of links split, etc.). Plasmin's action on proteins is not as extensive as that of trypsin[34] nor will it act upon all of the proteins susceptible to tryptic digestion.

Streptokinase is the most effective therapeutic agent currently available for enhancing the resolution of fibrin in closed body cavities containing traumatic or inflammatory effusions, and is considerably superior to proteolytic enzymes for this purpose. Though this statement may be at variance with the known action of proteolytic enzymes on fibrin, in biologic situations, proteolytic enzymes are rapidly inactivated by the large amounts of proteolytic enzyme inhibitors invariably present. In contrast, the major attribute of streptokinase lies in its special fibrin-dissolving properties. Though classically the action of streptokinase in dissolving fibrin-containing exudates or clots has been attributed to the amount of plasmin activated in the body fluids, recent observations reveal this to be a relatively minor part of the effect.[35,36] The latter studies reveal that all fibrin deposits contain plasminogen within their interstices, and that the primary mechanism for the dissolution of fibrin deposits is the diffusion of a plasminogen activator into the fibrinous substance with resultant activation of the plasminogen system within the fibrin meshwork, followed by rapid fibrinolysis. For this reason, streptokinase, with its capacity for activating the intrinsic plasminogen of fibrin deposits, has "fibrinolytic" properties considerably in excess of that which may be accounted for by the plasmin content of body fluids. In addition, and in contrast to the rapid inhibition of proteolytic enzymes by naturally occurring humoral antiproteolytic substances, streptokinase is inactivated at a relatively slow rate (except in the presence of an excess of a specific antibody, antistreptokinase). Thus, in terms of duration of action and specificity for lysing fibrin, streptokinase enjoys distinct advantages over proteolytic enzymes and is the more useful agent for fibrin dissolution in patients. On surface wounds, however, where high levels of proteolytic activity can be sustained because of the lack of inhibitory exudates, little advantage accrues to the use of streptokinase, particularly in the presence of pathologic substrates insusceptible to the action of plasmin (e.g., excess respiratory mucus, burned skin, necrotic connective tissue).

Streptodornase (streptococcal desoxyri-

*Varidase.

bonuclease)* acts directly upon desoxyribonucleic acid (DNA), rapidly depolymerizing this highly complex substance into small units.[17,19,38] The activity of streptodornase is enhanced by the presence of Mg^{++} or other divalent metallic ions, and inhibited by the presence of substances, such as citrate, which form complexes with the metallic cofactor.[17] A unit of streptodornase activity refers to that amount of enzyme which under standard conditions reduces the viscosity of a DNA solution by 1 viscosity unit in 10 minutes.[39] Inflammatory exudates contain little inhibitory activity to the action of streptodornase except for the presence of a specific antibody (antistreptococcal desoxyribonuclease), the titer of which may be significantly elevated following recent streptococcal infections or previous streptodornase therapy.[40,41]

The amounts of streptokinase-streptodornase mixtures suggested for local use, when properly applied, are usually sufficient to produce significant enzymatic effects in vivo,[13,17] and their usefulness as therapeutic agents previously has been reviewed.[14,31,42] Unfortunately, the use of streptokinase-streptodornase mixtures, particularly where they are most suited, has been restricted by the high incidence of pyrogenic reactions and locally irritating effects. The latter considerations are of little significance under conditions where there is an adequately established drainage to the exterior, e.g., in surface wounds, in the presence of open sinus tracts or surgical drainage. Under these latter circumstances, pyrogenic reactions are infrequently observed, and the local irritating effect of the enzyme preparation is not an undesirable feature. Indeed, it has been suggested that this irritating effect contributes significantly to the débridement by replacing the dead, dying, and degenerating cells with large numbers of fresh, viable phagocytes and other new immune serum forces.[14,18] However, in diseases involving closed body cavities or spaces containing clotted blood, thick pus, heavy layers of surface fibrin, or loculated effusions, where conservative medical treatment with streptokinase-streptodornase mixtures should prove a most efficacious adjunctive procedure, the pyrogenic and irritating effects of the preparation not infrequently complicate therapy.

The basis for these pyrogenic reactions[43] is difficult to assess since a number of factors may contribute: bacterial pyrogens in the enzyme preparation, endogenous pyrogen released from the enzyme-induced inflammation (pyrogenic reactions are more likely to occur where the enzyme preparation provokes an intense local inflammatory reaction), and the absorption of breakdown products resulting from the enzyme action. Of these various factors, we suspect the presence of pyrogenic material in the currently available streptokinase-streptodornase mixtures, and endogenous pyrogen released from the enzyme-induced inflammatory reaction as being of primary importance in the development of the febrile reaction.

The local irritating effects of streptokinase-streptodornase mixtures, when injected into closed spaces, also may complicate therapy since a fresh inflammatory exudate rapidly accumulates. This fluid accumulation has made frequent and repeated drainage of the involved area mandatory; delay in carrying out drainage, especially in small or relatively fixed closed spaces (e.g., eye, spinal canal, etc.) occasionally has been associated with serious consequences.[44,45] The local inflammatory response to the streptococcal enzyme mixture varies considerably among patients and is usually the more striking when applied to areas where the previously existing inflammatory reaction is of relatively low grade or absent.

*The use of the term streptodornase has resulted in some semantic problems. Originally introduced as an abbreviation for streptococcal desoxyribonuclease, it has become apparent that there are 3 serologically distinct desoxyribonucleases,[37] designated A, B, and C, as well as a number of additional enzymes capable of extensively degrading DNA into free purine bases and pyrimidine desoxyribosides.[19] Since the activity of these preparations is measured in terms of desoxyribonuclease activity, it seems appropriate that the term streptodornase be restricted to connote the total desoxyribonuclease activity rather than designating any specific enzyme.

In our personal experience, patients with tuberculous involvement of serous membranes (pleural empyemas, meningitis, pericarditis) often have an exaggerated inflammatory response to the local instillation of the streptokinase-streptodornase mixture. Though the nature of the inflammatory response has been investigated,[18] the cause is obscure; our impressions suggest that there is a delayed sensitivity-like reaction to one or more streptococcal proteins in the complex mixture.

2. *Trypsin.* Crystalline trypsin preparations of beef pancreatic origin are available commercially* in powder or ointment form for débriding purposes, and usually are standardized in terms of their proteolytic activity. Trypsin directly hydrolyzes a large number of naturally occurring proteins; it does not affect living cells nor require any cofactors, and its action on denatured proteins is usually more extensive than on native proteins. Trypsin has certain theoretical advantages over streptokinase for surface wound débridement since it does not require additional factors for its action; acts upon a greater number of proteins than plasmin and degrades them more extensively. Moreover, unlike the streptokinase-plasmin system, trypsin is capable of hydrolyzing the protein moiety of the respiratory mucins, thereby rendering respiratory secretions much less viscid.[46-48] Despite these theoretical advantages, the locally irritating effects of trypsin preparations on the respiratory mucosa have often limited their usefulness in maintaining a respiratory toilet,[49-51] and a fairly extensive trial with trypsin as a surface débriding agent has resulted in effects analagous to those described for streptokinase-streptodornase preparations.[30,31] Trypsin, when injected into closed spaces, appears to be of lesser value as a débriding agent; these preparations not only incite a high incidence of local inflammation and systemic pyrogenicity, but the enzyme is rapidly inactivated.

3. *Chymotrypsin.* Chymotrypsin, the other major proteolytic enzyme of the pancreas, is also available commercially* in crystalline form for local use on infected wounds either alone or in combination with trypsin. The preparations are of beef pancreatic origin and usually standardized in terms of their proteolytic activity. Though chymotrypsin acts upon different bonds in proteins than does trypsin or plasmin, its spectrum of activity on whole proteins is somewhat similar to that of trypsin. When it is combined with trypsin, proteins are more extensively hydrolyzed than with either enzyme alone. In general, as much can be accomplished with chymotrypsin as a local débriding agent as with trypsin and its usefulness is limited by similar considerations. Though recent reports[52,53] have stressed the usefulness of topically applied α-chymotrypsin as an adjunct to the surgical removal of cataracts, more extensive experience is required before a final evaluation can be made.

4. *Papain.* A partially purified preparation of the powerful plant cathepsin, papain, standardized in terms of proteolytic activity and combined with urea as a denaturing agent, is available† for wound débridement. Papain acts on a wide variety of proteins and its activity can be considerably enhanced by the addition of cysteine or other reducing agents. It enjoys one distinct practical advantage over the previously described preparations; the enzyme is active over a wide pH range (pH 3 to 9), thus eliminating the need for careful buffering. At low pH, papain is capable of digesting collagen.[54] Though occasionally papain preparations have been used in acetic acid solutions to digest collagenous tissue, the success of this method has not been established. Unfortunately, clinical investigation with more purified papain preparations has been limited[55] despite advantages in cost, spectrum of activity, and pH considerations which might allow it to be developed into

*Tryptar, Parenzyme (ointments contain chymotrypsin as well)

*Chymar (ointment contains trypsin as well).
†Panafil.

a most practical agent for surface débridement. Papain preparations should not be injected into closed spaces; in a few instances where this has been done, harmful effects occasionally were observed.[56]

Interpretative therapeutic evaluation. The commercially available proteolytic enzyme preparations including streptokinase-streptodornase mixtures are active as enzymatic débriding agents when applied directly to infected surface wounds or to infected areas where there is open and adequate drainage to the exterior. Streptokinase-streptodornase preparations are the most useful in the presence of thick purulent exudates or where heavy deposits of fibrin are present. Otherwise, when used carefully and with proper attention to detail, all of the current preparations have been used successfully to débride dirty, infected wounds, except in instances where large amounts of dead skin slough or necrotic connective tissue are present. Purified papain preparations eventually may prove to be the most practical for surface débridement.

Streptokinase-streptodornase preparations are the agents of choice for liquefying clotted blood, loculated effusions, and purulent exudate in closed body cavities; their use for this purpose is attended by demonstrable effects, often of therapeutic benefit to the patient. However, a significant incidence of pyrogenic and inflammatory reactions to the locally administered enzyme mixture has limited its usefulness since the therapeutic procedure may be complicated by the patient's discomfort and the need for frequent and repeated drainage. It is likely that these undesirable effects are due to impurities in the current preparations (approximately 12 per cent of the current material is streptokinase) and not to the specific enzymes themselves or their enzymatic effects. This emphasizes the need for a highly purified streptokinase-streptodornase preparation. In a few selected instances we have injected highly purified streptokinase intrapleurally in sufficient dosage to produce therapeutic fibrinolysis; this was not associated with either a pyrogenic or local inflammatory reaction and the need for repeated drainage was obviated. Further studies with highly purified preparations are indicated for they could extend considerably the usefulness of this type of therapy.

Further exploitation of the concept of local enzymatic débridement requires a number of additional developments. These include better and simpler methods for achieving direct and sustained contact of a watery solution of the enzyme with the affected tissues, further purification of some of the preparations in current use, additional methods for reducing their irritating and pyrogenic effects, combined laboratory and clinical studies aimed at clearly defining the indications for each preparation, and the development of enzymes capable of lysing the insoluble constituents of dead tissue not attacked by the available preparations. Investigation is proceeding along the latter line; enzymes under current study include ficin,[57,58] Clostridial collagenase,[59] pancreatic desoxyribonuclease,[51,60,61*] bovine plasmin,[61] and human plasmin.[65,66]

III. Proteolytic enzymes as anti-inflammatory agents

The use of proteolytic enzymes as anti-inflammatory agents remains controversial. Empirical observation rather than scientific data still dominates the subject.

Experimental background. In 1952, Innerfield, Schwarz, and Angrist[67] reported that intravenously administered trypsin induces lytic effects upon artificially formed intravascular thrombi in rabbits and dogs. In extending these observations to patients, the authors noted that over 94 per cent of the patients treated with trypsin intravenously enjoyed a rapid subsidence of all signs and symptoms of acute inflammation.[68] The results were surprising, since many of the treated patients were suffer-

*Pancreatic desoxyribonuclease also is being investigated for systemic use by intravenous administration[62]; preliminary observations suggest that it may prove useful in the treatment of pneumococcal meningitis[63] and pulmonary abscesses.[64]

ing from such diverse diseases as acute rheumatoid or gouty arthritis, atypical virus pneumonia, rheumatic carditis, acute ulcerative colitis, etc., where intravascular thrombosis is not suspected of playing a major part in the morbidity. Furthermore, in the individuals with thrombotic disease, e.g., thrombophlebitis, effects were observed a few hours after institution of the trypsin treatment though evidence of any thrombolysis was considerably delayed. To reconcile these observations, particularly since others were unable to confirm a thrombolytic action of trypsin in vivo,[69-73] it was suggested that the effects were produced by an anti-inflammatory action of trypsin rather than by an effect on intravascular thrombi. Experimental confirmation was provided by Innerfield,[74] who administered chymotrypsin and streptokinase intravenously to rabbits containing a Sotradecol-induced thrombus in the marginal ear vein and reported the occurrence of a striking reversal of perivascular and interstitial inflammation without significant change in the thrombus itself. These observations appeared to be in accord with previous studies by Martin, Brendel, and Beiler[75] who noted that the production of egg white edema in rats could be partially inhibited by the parenteral administration of trypsin, chymotrypsin, streptokinase, and prolase B, and by Adamkiewicz, Rice, and McColl,[76] and Cohen, Graff, and Kleinberg,[77] who reported a similar inhibition of kaolin-, yeast filtrate-, or dextran-induced edemas in normal and adrenalectomized animals. Additional evidence for an anti-inflammatory effect was presented by Gordon and Ablondi,[78] who significantly reduced a mustard oil inflammation of the rabbit eye by intravenously administering streptokinase, and by Miechowski and Ercoli,[79] who inhibited a number of inflammatory responses in several animal species by administering chymotrypsin. The evidence presented in these studies indicated that the anti-inflammatory effect of proteolytic enzymes was mediated by a mechanism independent of that noted with adrenal steroids.

The mechanism of this anti-inflammatory effect remains an enigma. Studies demonstrating the effect have been restricted to grading the amount of inflammation observed either grossly or by microscopy; no studies are available on the specific alterations induced under controlled conditions on the development or evolution of the inflammatory response. As a consequence, all the interpretations remain of a speculative nature. Originally it was reasoned that the administration of enzymatic factors capable of influencing fibrin resorption was enhancing the rate of dissipation of the inflammatory exudate. Though this may be true with streptokinase, where the effect in animals has been observed only in the presence of enhanced circulating fibrinolytic activity,[78] the trypsin and chymotrypsin effect was observed in the absence of increased circulating fibrinolytic activity. Current speculation is that these enzymes act by dissolving or depolymerizing the impacted or obstructing macromolecules enmeshed in the connective tissue, thus increasing the permeability of the inflamed area and hastening the resolution of the exudate. No evidence is available to support this view which presupposes that the enzymes are delivered intact by the circulation to the inflamed area and ignores the powerful antiproteolytic activity of the serum. Bastian, Hill, and Ercoli[80] attempted to but could not demonstrate by very sensitive techniques increased levels of circulating trypsin or chymotrypsin following the intramuscular administration of relatively large amounts of these enzymes, and concluded that the anti-inflammatory effect noted following their injection is probably not mediated by systemic absorption.

Clinical observations. Intramuscular and buccal preparations of trypsin, chymotrypsin, and streptokinase-streptodornase* are in clinical use as anti-inflammatory agents in the management of human disease. Much of a recent symposium on the clinical use

*Parenzyme (aqueous, in oil, buccal), Chymar (aqueous, in oil, buccal), Enzeon, and Varidase (intramuscular, buccal).

of proteolytic enzymes[81] was devoted to a description of the gratifying results achieved with intramuscular therapy in such widely diverse situations as thrombophlebitis, lumbosacral strain, the prevention of postcraniotomy cerebral edema, and the hastening of absorption of hematomas occurring after football injuries. Most of the clinical observations with the parenterally administered proteases now has been reduplicated with the buccal preparations; a recent article by Innerfield[82] reviews this aspect of the subject.

Considering that there is experimental evidence to support the clinical observations, why is the value of this type of therapy questioned? Those who remain skeptical cite the following criticisms:

1. Lack of experimental c supporting a reversal of an established lammation. The experimental models used in stablishing the anti-inflammatory effect of the proteolytic enzymes all involve the parenteral administration of the enzyme before the local application of the irritant. The prevention of an inflammatory effect may be quite different or much more readily achieved than its reversal. For example, in the studies of Gordon and Ablondi,[78] increased circulating fibrinolytic activity was established in the rabbit before the application of the mustard oil to the eye. Under these circumstances, the fibrin deposited in the inflammatory zone already contained the enzymatic factors capable of influencing its rapid resorption. This is quite different from the dissolution or breakdown of fibrin and other macromolecular aggregates which have been deposited previously and must now be attacked from without. In the studies of Kleinfeld and Habif[83] where trypsin and chymotrypsin were given parenterally through various injection sites, after the induction of a granuloma p ich in rats, no effect attributable to the en ies could be demonstrated.

2. Lack of correlation betwee ose level employed in man and that required o demonstrate an anti-inflammatory effect in animals. The minimal effective dose required to demonstrate a significant anti-inflammatory effect in animals has varied depending upon the type of irritant used; the most sensitive model responded to 0.8 mg. of trypsin per kilogram; the most resistant to 5.0 mg. of trypsin per kilogram. In most of the experiments reported, the minimal effective dose was 2.0 mg. per kilogram. The dosage employed in clinical studies has ranged only from 0.08 to 0.25 mg. per kilogram. Furthermore, in the animal studies, the doses cited usually represent parenteral injections of aqueous enzyme, the required dosage for which is lower than for oily suspensions or for buccal tablets[84]; in the studies in humans the values represent trypsin in oil preparations and more recently trypsin in buccal tablets. It is impossible to estimate how much trypsin is absorbed from these tablets since, even with aqueous preparations at the dose levels employed, increased circulating tryptic activity cannot be demonstrated.[80] Similar calculations can be made for chymotrypsin; the amounts used clinically are approximately one-tenth to one-twentieth those required in animals, and the preparations used (particularly the buccal tablets) may result in variable or incomplete absorption. Here too, as with trypsin, increased enzyme activity in the circulation cannot be demonstrated.[80] In the case of streptokinase, it is more difficult to make comparisons. Several of the reports refer to the streptokinase dosage in animals in milligrams per kilogram, a term which has little meaning since preparations can vary greatly in their activity depending upon their state of purity. Furthermore, it is recognized that the ability of streptokinase to activate the fibrinolytic enzyme system of various animal species varies greatly. Where suitable observations have been made in animals, the minimally effective dose required to demonstrate an anti-inflammatory effect was sufficient to produce a marked enhancement of circulating fibrinolytic activity.[78] In the clinical studies, the doses employed are very small in comparison with those used in animals but, more importantly, no enhancement of

circulating fibrinolytic activity has been demonstrated. Though the use of buccal tablets containing streptokinase-streptodornase has been reported to result in the subsequent appearance of some antibody in the majority of treated patients,[85] the observations have little significance in quantitating absorption.

3. *Most of the clinical observations are purely empirical; where controlled studies have been performed, the results are unconvincing.* Of the many reports on the use of enzymes for their anti-inflammatory effect, only two were designed so as to allow for a statistical evaluation.[86,87] Kryle, Arnoldi and Kupperman[86] studied, by means of the double blind technique, the effect of a 5 day course of intramuscularly administered trypsin in sesame oil on all types of acute thrombophlebitis occurring in 82 patients. The patients were selected at random from the various services of a large county hospital. Regardless of the special treatment, i.e., trypsin or placebo, all the patients were treated with bed rest and other conventional procedures. All patients with superficial phlebitis received continuous wet soaks and all patients with moderate and severe thrombophlebitis, as well as mild deep phlebothrombosis, received anti-coagulants in full therapeutic doses. After the patients were observed for 5 to 7 days, the effect of the treatment was evaluated. The authors concluded that their results clearly indicated the value of trypsin in the treatment of this disease. The validity of their observations may be seriously questioned, however, since the control group did much more poorly than the usual clinical experience with this entity (63 per cent showed no improvement or were worse).

Norman and Kadull[87] have recently concluded a controlled double blind study on the anti-inflammatory effect of buccal streptokinase. A group of 200 volunteers were given a standard inflammatory stimulus in the form of a subcutaneous injection of an alum-precipitated anthrax vaccine known to produce a 25 per cent rate of local allergic reactions. The patients received 40,000 units of streptokinase in buccal tablets or indistinguishable placebos on the same day as they received the anthrax vaccine. The incidence of reactions was identical in both groups and it was impossible to demonstrate any anti-inflammatory effect in this study. Thirty thousand units of streptokinase in buccal tablets or an identical placebo then was administered daily for 3 days to an additional 100 volunteers. On the third day they were given the anthrax vaccine. Again, no difference in the incidence or severity of the reactions was noted among the two groups.

Interpretive therapeutic evaluation. Considering the data available, we believe that the evidence supporting the existence of an anti-inflammatory effect in man for such enzymes as trypsin, chymotrypsin, and streptokinase (at the dose levels and methods of administration in current vogue) is still unsatisfactory and that the burden of responsibility for proving this effect remains upon those who advocate their use.

IV. Therapeutic thrombolysis

The high incidence of thromboembolic disease in man, especially in the elderly, with its heavy concomitant mortality and crippling morbidity, has stimulated work toward the therapeutic goal of in vivo thrombus or embolus dissolution by enzymatic means. Chief interest has focused on attempts to utilize the plasminogen-plasmin system for therapeutic purposes because of its key physiologic function in the lysis of in vivo fibrinous deposits. Subsidiary interest has been shown in other proteolytic enzymes (capable of lysing fibrin and available in crystalline form), which have been subjected to experimental evaluation and clinical trial.

Until recently the field of clinical thrombolysis was in a chaotic state since clear physiologic and therapeutic concepts were lacking and much of the evidence relating to therapeutic efficacy rested on the insecure basis of uncontrolled clinical trial. Though at present substantial concepts of real promise are emerging, this section will

be chiefly concerned with a review of the problem as a whole rather than a description of actual human therapeutic achievement, particularly since the latter is best considered as still in an experimental stage.

The evidence will be considered in the following order: (1) biochemical considerations, (2) animal experiment, (3) development of physiologic concepts governing thrombolysis, (4) toxic properties of various thrombolytic substances, and (5) clinical experience.

Biochemical considerations.* A proenzyme, plasminogen, is a constituent of normal plasma; on its activation either spontaneously[89] or in the presence of specific activators,[80] a proteolytic enzyme, plasmin, is formed. Plasmin (molecular weight 106,000) has a spectrum of proteolytic activity similar to but not identical with that of trypsin; among its substrates are fibrin, fibrinogen, accelerator globulin, some components of complement, ACTH, growth hormone, and glucagon. Casein is a convenient and suitable substrate for its assay[91] and biochemical activities discussed later will be expressed in casein units.[92] Despite its widespread use, fibrin is an unsuitable substrate for plasmin assay in biologic systems and many errors may be attributed to its employment in such situations.[36]

Plasma contains powerful and effective proteolytic enzyme inhibitors; these constitute an essential safeguard against the appearance of excessive states of plasma proteolytic activity. If these physiologic mechanisms prove inadequate and a state of excessive plasma proteolysis occurs, the manifestations of "fibrinolysis" as a disease state become apparent.

The value for serum trypsin inhibitor is 1.03 ± 0.13 mg. trypsin per milliliter of serum,[93] and the value for chymotrypsin inhibitor roughly the same.[94] Antiplasmin values average 5.1 casein units per milliliter of plasma, which may be contrasted with plasminogen values of 3.5 casein units per milliliter.[95]

*For a more detailed discussion see references 36, 88.

Plasma inhibitory substances constitute a severe impediment to the "therapeutic" use of proteolytic enzymes since, though the precise type of union between enzyme and inhibitor is the subject of controversy, enzyme-inhibitor combination occurs rapidly.

Animal experiment. There is abundant experimental evidence to document the finding that the intravenous infusion of proteolytic enzymes or plasminogen activators will, under appropriate circumstances, resolve thrombotic arterial or venous lesions produced in animals. However, the differing experimental conditions used by investigators have hitherto tended to obscure the real significance of their findings. Foremost among these difficulties has been the confusing species variability of animal plasminogen in its behavior to streptokinase (the plasminogen activator used by virtually all investigators). Almost equally troublesome has been the difficulty attendant upon inadequate assay of experimental preparations for the separate biochemical moieties; plasminogen activator and proteolytic enzyme. We believe that the available evidence indicates: (1) that the mechanisms by which proteolytic enzymes and plasminogen activators exert a thrombolytic effect are different (vide infra), (2) that while plasminogen activators have been proved to be effective thrombolytic agents in vivo, the evidence in favor of proteolytic enzymes acting similarly, except under very restricted conditions, is inadequate, and (3) that the secondary effects of proteolytic enzyme infusion are of a sufficiently grave character to render their therapeutic use impracticable.

Johnson and Tillett[96] were the first to show that the intravenous infusion of streptokinase* into rabbits would cause lysis of experimentally induced thrombi situated in the marginal ear vein. This important investigation has been the model for others and a high degree of success has been achieved in the dissolution of experimental

*Varidase was the preparation employed and has been used by most investigators in this field.

thrombi situated in several anatomic sites and in several species when either streptokinase, streptokinase-activated or urokinase-activated plasmin is used.† Unfortunately, controversy has surrounded the interpretation of experiments involving the use of streptokinase-activated or urokinase-activated "plasmin" as thrombolytic agents. To the original workers[97-99] it has always seemed certain that the results could be attributed to the action of exogenous plasmin lysing the thrombus and no other interpretation was allowed for in the experimental design. To others[70] it has appeared probable that the plasminogen activator content of the "plasmin" preparations was of major importance. But the original "plasmin" preparations were never adequately assayed or characterized and, though inferences can be made concerning their composition, certainty cannot be achieved. Assays have, however, been made on certain more modern "plasmin" preparations and their composition is discussed later.

The administration of proteolytic enzymes to animals suffering from experimental thromboembolic disease has not been followed by predictable or uniform effects upon the thrombus; while Innerfield, Schwarz, and Angrist[67] reported that trypsin acted as a potent thrombolytic agent in rabbits and dogs, Taylor, Overman, and Wright,[71] after thorough study, were unable to confirm this finding in the rabbit. They further drew attention to the serious degree of intravascular clotting that occurred following trypsin administration, a finding previously the subject of extensive study.[100-102] Other investigators failed to confirm the claim that trypsin was an effective thrombolytic agent in the dog.[69,70,72,73]

Since chymotrypsin does not produce intravascular clotting and in fact produces a coagulation defect upon intravenous injection in the animal, it would appear to be a preferable enzyme to trypsin, where intravascular thrombolysis is desired. A group of investigators[70] have reported that chymotrypsin produces a powerful thrombolytic effect on arterial lesions in the dog; however, Tagnon[103] states, without details, that in his hands chymotrypsin produced no thrombolytic effect upon similar lesions.

A study by Sherry, Titchener, Gottesman, Wasserman, and Troll[70] utilizing an experimental thrombus in the dog shed considerable light uopn the mechanisms of in vivo thrombolysis. Whereas the administration of streptokinase produced lysis of experimental arterial thrombi with only minor coagulation disturbances and minimal plasma proteolytic activity, trypsin in doses sufficient to sustain high levels of proteolytic activity and to produce gross changes in the coagulation system proved to have no thrombolytic effect. However, chymotrypsin administered in high dosage (12-30 mg. per kilogram) caused lysis of a high proportion of the experimental thrombi even though the degree of plasma proteolytic activity produced was no greater than with trypsin and disturbance of the coagulation mechanisms was less.* The importance of these findings lay in the clear demonstration that clot-lysing ability and proteolytic activity of plasma were independent variables. In a later paper[107] this group demonstrated that, following peritoneal trauma, the administration of plasminogen activator to dogs would prevent or reduce the degree of postoperative peritoneal exudation and organization.

Ambrus, Ambrus, Back, Sokal, and Collins[98] made an extensive comparative study

*"Plasmin" preparations used for these studies were grossly contaminated with streptokinase and evidence that the observed effects were due to plasmin action are lacking. It is the reviewer's opinion that contamination of the "plasmin" preparations with plasminogen activator exerted a decisive influence on the reported results.

†Similarly urokinase-activated "plasmin" may have exerted a thrombolytic action because of its UK content.

*Certain interpretations made in this paper with regard to the respective actions of trypsin and chymotrypsin on coagulation moieties may require revision in accordance with more recent findings.[104-106] It is now known that the products of fibrinogen breakdown produced by proteolytic digestion cause a coagulation defect due to defective fibrin polymerization.[106] This defect causes error in certain standard coagulation assays and the degree of error produced is different when fibrinogen is digested by trypsin or chymotrypsin as dissimilar breakdown products are formed.

of the thrombolytic actions of crude pancreatic protease, trypsin, chymotrypsin, carboxypeptidase, papain, ficin, streptokinase, chloroform-activated plasmin, and various preparations of human plasmin (mixed with streptokinase). The test thrombi (made in the dog) were labeled with I^{131} fibrinogen and thrombolysis rates determined by radioactive measurement over the thrombus site. They reported that only the "plasmin"* preparations showed in vivo fibrinolytic activity at nontoxic dose levels. With similar methods[108] it was later found (as would be expected on general pathologic grounds) that response to "plasmin" infusion was diminished with increasing age of the thrombus and that little response was obtained with thrombi older than 72 hours.

An important consideration when employing enzymatic thrombolytic therapy concerns the final state of infarcted tissue after the blood supply has been restored. Two groups of investigators,[109,110] using the dog, have observed the effects of lysing a thrombus obstructing a coronary artery. Both believed that the area of final infarction was reduced by such therapy and neither noted any unusual histologic lesions secondary to the therapy. Though in neither instance did the animal model approximate the sitation commonly found in the human, the results are of an encouraging nature.

A yet unsolved problem concerns the effect of thrombolysis in the cerebral circulation. In an excellent study the Mayo group[111] have shown that under certain circumstances the administration of anticoagulants to dogs suffering from obstructive cerebral vascular disease may lead to hemorrhage in the infarcted area. Though the clinical importance of these observations is yet uncertain, they convey the warning that, from a functional viewpoint, infarction of the brain may differ in important respects from infarction in other organs.

*Streptokinase and human plasmin act synergistically as powerful activators of dog plasminogen and it must be regarded as doubtful whether the "plasmin" preparations acted therapeutically because of their proteolytic potency.

Mechanisms of physiologic and therapeutic thrombolysis. Though both proteolytic enzymes and plasminogen activators have been used to produce thrombolytic effects in animals, the secondary effects produced by these agents are different. Whereas proteolytic enzymes used in sufficient dosage to exert a thrombolytic action invariably produce serious disturbances in coagulation mechanisms, thrombolytic phenomena produced by plasminogen activators may occur without such disturbance.

While the production of marked disturbance to physiologic homeostasis is unimportant in the experimental animal provided that thrombolysis is achieved, different considerations are paramount in the human. Consequently, the transfer of impressive experimental results obtained in animals to the realm of human therapeutics has involved investigation of physiologic mechanisms largely disregarded in or partly irrelevant to animal studies. Thus the problem in man has been twofold, first, to induce satisfactory thrombolytic states but second, and of equal importance, to restrict disturbances to homeostatic mechanisms to the minimum.

Particularly pertinent to the solution of this dual problem have been investigations concerning normal thrombolytic mechanisms and abnormal states of "fibrinolysis" (excessive plasma proteolysis) in man. Clearly caution in the use of experimental tools has been mandatory for it has been the lot of many physicians to treat or attempt to treat the grave consequences of "fibrinolysis" as a disease state in the human.

The knowledge that plasmin would digest fibrin has led most investigtors to assume that, under certain circumstances, plasminogen is activated in body fluids and the plasmin thus formed digests fibrin. This concept has been held to justify not only the administration of "plasmin," but also such other proteolytic enzymes as trypsin or chymotrypsin as therapeutic agents. Many investigators, however, including Ratnoff,[112] Mullertz,[113,114] and Sherry,[115] have cited

evidence casting considerable doubt on the validity of this simple hypothesis, which even at the time of its inception was inadequate to explain the known facts.

The new hypothesis. Apparently unassailable evidence has now been advanced[35,36] to indicate that exogenous plasmin action is of purely secondary and negligible importance in thrombolytic mechanisms: the chief and probably only mechanism of in vivo thrombolytic action occurs through diffusion or adsorption of a plasminogen activator to a thrombus, activation of intinsic plasminogen contained within the interstices of the thrombus and consequent thrombolysis. This concept developed from a study of enzymatic factors influencing the lysis of preformed human plasma clots labeled with I^{131} fibrin immersed in both artificial test systems and biologic fluids.[35] Whereas plasmin (without contaminating plasminogen activator) in amounts greater than were ever found in plasma (after total plasminogen activation had occurred) produced a negligible thrombolytic effect but considerable disturbance to coagulation moieties, plasminogen activators in low concentration were highly effective thrombolytic agents. Moreover clot lysis rates in plasma treated with streptokinase or urokinase were shown to be a function of both activator concentration and clot plasminogen concentration, as would be expected were activation of intrinsic clot plasminogen by exogenous activator the responsible mechanism. Also in accord with this mechanism were the observations that thrombolysis rates could be strikingly inhibited by removing plasminogen from the preformed clots or by adding an activator inhibitor (epsilon aminocaproic acid) to the test plasma.

Confirmatory to the general hypothesis that physiologic thrombolysis occurs as a consequence of plasminogen activator release rather than as a consequence of exogenous plasmin action have been the following demonstrations: (1) the thrombolytic activity of plasma developing after exercise, therapeutic electroshock, drug administration, and other stress situations is due to the presence of plasminogen activator and not to the presence of plasmin[116]; (2) low levels of thrombolytic activity assayed in plasma from normal unstressed adults exhibit the biochemical stigmas of plasminogen activator and not of plasmin[117]; (3) the thrombolytic and proteolytic activities of plasma that develop in various conditions are independent variables[95,118]; and (4) thrombolytic activity may occur without detectable alteration of plasma fibrinogen, plasminogen, or antiplasmin concentrations.[116,117]

The establishment of this present hypothesis has allowed differentiation between thrombolysis or thrombolytic activity as a normal physiologic function, and hyperplasminemia as a pathologic state frequently associated with the clinical manifestations of increased proteolysis ("fibrinolysis" as a disease state). Thus the physiologic function of plasminogen, contained in plasma, appears to be that of endowing thrombi or other fibrinous deposits with sufficient proenzyme to mediate their subsequent lysis. The pathologic manifestations of plasma "fibrinolysis" formerly regarded as of crucial importance appear now to be merely an incidental consequence of the plasma transport mechanism for plasminogen and irrelevant to the physiologic role of plasminogen in thrombolysis. Extended presentations of these concepts have recently been published[36,119,120] and these merit study because of their implications not only with regard to matters of physiologic interest but also because of their therapeutic implications.

Systemic effects of intravenously administered proteolytic enzymes and plasminogen activators. A crucial parameter for any drug concerns its therapeutic ratio which in this instance will be strictly defined as the dose of drug producing a beneficial effect against the dose of drug producing a deleterious effect, whether or not such a deleterious effect is clinically apparent.

Study of this parameter has been extremely difficult in the case of proteolytic

enzymes or plasminogen activators for, on account of difficulties in purification, the chief parameters studied in the past have sometimes been those related to contaminating impurity contained in various preparations; only recently has it been possible to study the toxicity of the materials themselves.

On the basis of simple considerations concerning ratios of enzyme-substrate specificity and the probable kinetics of in vivo enzyme-substrate competition, it can be predicted that all the proteolytic enzymes so far tested in vivo will have a therapeutic ratio of less than one. Such calculations exclude such unfavorable special properties of particular enzymes as those of trypsin on the coagulation mechanism.

Survey of the animal data, cited in the last section, suggests that such calculations are valid. The administration of proteolytic enzymes in sufficient dosage to exert thrombolytic action has invariably been associated with undesirable effects secondary to abnormal degrees of plasma proteolytic activity: the coagulation system has proved to be particularly vulnerable to such biochemical insult. Though infusion of the proteolytic enzyme "plasmin" has been claimed to exert preferential fibrinolytic effects,[121,122] the evidence in favor of this hypothesis can only be characterized as inadequate since *large amounts of activator were included in the preparations.*

Present evidence concerning the effects of proteolytic enzyme infusion in man is scarcely relevant to the problem of assessing potential toxicity. First in the case of trypsin (see clinical section), homeopathic dosage has been employed because of side effects, and the demonstration of tryptic activity in plasma not accomplished. Second in the case of "plasmin" (see clinical section), it can reasonably be doubted whether the reported results refer to the effect of plasmin infusion or the effect of plasminogen activator infusion.

Plasminogen activators. To date the only plasminogen activator prepared in sufficient quantity for adequate clinical trail has been streptokinase. Early reports on the intrapleural use of streptokinase (as Varidase) drew attention to symptoms of chills, fever, and malaise produced by the material; patients became immunized, developing high titers of streptokinase antibody over a 1 to 4 week period. Similar reactions, though of greater degree and associated with hypotensive episodes, severely limited intravenous dosage, even when antipyretic and antihistaminic drugs were administered.[123]

Later the intravenous use of more highly purified streptokinase preparations[124] biophysically homogenous but containing impurity on immunochemical examination (potency 600 Christensen units per microgram N) was shown to result in a greatly lessened incidence of toxic reactions.[125,126] Recently streptokinase* of the same or slightly higher potency, but showing less impurity on immunochemical examination, has received extended clinical trial.[118,127-131]

The sole toxic effects recorded have been due to the action of streptokinase as a plasminogen activator (vide infra) and neither fever† nor hypotension has been observed, even when relatively enormous doses (2 to 6 million streptokinase units) have been administered by intravenous infusion over a 30 hour period.[95] Consequently it can be concluded that previous findings with regard to streptokinase toxicity refer to insufficiently purified streptokinase rather than to streptokinase itself.

Though much difficulty is currently being experienced in the large scale purification of streptokinase suitable for clinical use, encouraging progress has recently been made by more than one pharmaceutical company and it is to be hoped that sufficient material for large scale trial may soon be made available.

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*Supplied for clinical investigative purposes by Lederle Laboratories, Pearl River, N. Y. Details of manufacture are not available and the supply of material has been restricted.

†Johnson and McCarty,[131] using streptokinase bearing the same lot number and similar material bearing different lot numbers, have noted occasional mild fever following streptokinase infusion.

Systemic effects due to plasminogen activator excess. A severe hemorrhagic diathesis may complicate "fibrinolytic" states associated with disease. Etiological factors responsible for such phenomena have not been completely evaluated (for review see reference 36), but essentially sudden release of plasminogen activator into the circulation and some second factor (such as concomitant release of thromboplastic material) are necessary precipitating events. Biochemically hyperplasminemia is found and plasma proteolysis causes fibrinogen breakdown. The pathogenesis of the coagulation defect is related to the presence of fibrinogen breakdown products which inhibit fibrin polymerization.[106] This coagulation defect may be so severe as to simulate, if standard coagulation assays are used, a state of afibrinogenemia: actually fibrinogen levels are found to be only moderately depressed if proper methods are employed.

Clearly the infusion of large amounts of plasminogen activator for therapeutic purposes carries the theoretical risk of inducing a coagulation defect similar to that found in disease states. There is [illegible] evidence, however, that though a coagulation defect does occur as a consequence of plasminogen activator infusion, its magnitude and consequences are not a bar to treatment with plasminogen activators. The reader is referred to the original papers[95,129] for details.

A corollary to this work on the coagulation defect developing during the state of hyperplasminemia is that infusion of plasmin, in amounts sufficient to influence thrombolytic mechanisms, will inevitably cause a coagulation defect of disastrous proportions. Similar considerations will hold for other proteolytic enzymes.

The immunologic problem. The most obvious difficulty involved in the use of streptokinase or streptokinase-activated plasmin in man has been one of immunologic origin. The intention to infuse a potent antigen of streptococcal origin, streptokinase, for long periods and at high dosage into a patient population showing various degrees of antibody response against this antigen[132] raises a problem both unique in the realm of therapeutics and also of fundamental interest in the field of immunology.

As with other human populations exposed to a random immunologic stimulus, streptokinase antibody concentrations are distributed as a log normal variable[126] and the dissociation constant for the antigen-antibody complex, though not accurately determined, is known to be small.[39] For these reasons and also because the immunologic status of the patient influences both early and late streptokinase plasma clearance rates,[133] dosage rates vary widely for individual patients (over a fifty-fold difference in initial dosage to produce the same biochemical effect).

Studies in patients using I^{131}-labeled streptokinase[133] have shed light on this problem and are also of interest with regard to the fundamental immunologic problem involved. From a practical standpoint, satisfactory methods for predicting individual patient dosage have been devel- [illegible][95,127,133] and to date allergic or anaphy- [illegible]actions have not been observed, [illegible]ghly purified streptokinase is [illegible][127-131] Nevertheless present satis- [illegible] must still be coupled with a degree of uncertainty as supplies of purified streptokinase have been restricted and clinical experience has been limited in extent, particularly with regard to the treatment of highly immunized patients (5 per cent of the population). Furthermore it is emphasized that the present results have been obtained with the use of highly purified nonpyrogenic streptokinase and the danger of hypersensitivity or allergic reactions must still be regarded as considerable if streptococcal preparations contaminated with other streptococcal proteins are administered. In this connection it is relevant to cite the work of Kellner and Robertson,[134] who noted the appearance of lesions resembling Aschoff bodies in the myocardium of rabbits to which large amounts of crude streptokinase had been administered.

The use of a presumably nonantigenic

plasminogen activator such as urokinase (the plasminogen activator present in normal urine) would avoid the present difficulties, but suitable preparations are lacking. However, despite the original rather daunting nature of the immunologic problem posed by the use of an antigen such as streptokinase in man, it is evident that there are grounds for restrained optimism and that difficulties may ultimately prove to be more theoretical than real.

The administration of streptokinase intravenously causes immunization of the patient, a reaction which, though symptomless, may preclude re-treatment if thromboembolic disease recurs. This phenomenon is short lived since serial antibody assay on treated patients has shown the duration of this reaction to be only 3 to 6 months, at the end of which time antibody levels have declined to normal or near normal levels.[95]

Clinical experience.

Trypsin. Intravenously administered trypsin, despite the unsatisfactory and contradictory evidence derived from animal experience, has received a fairly extensive clinical trial as a thrombolytic agent in man. Though initial clinical reports were encouraging,[68,135-138] the lack of proved thrombolytic activity in vivo,[69-73] the hazard of induced clotting in vivo,[71,100-102] and the frequent production of local thrombophlebitis have resulted in its abandonment. Moreover, a former proponent of its use[74, 82,139] has recently withdrawn his original claim that intravenous trypsin administration exerted a thrombolytic action in the human and attributed his earlier clinical claims to an alleged anti-inflammatory action (see earlier section).

*Plasmin.** Numerous investigators[98,122, 140-155] have tested the effects of plasmin infusion in man and the majority have reported encouraging results in the treatment of patients suffering from selected forms of thromboembolic disease. However, review of the earlier data is difficult because the plasmin preparations used by the various workers were neither adequately characterized nor properly assayed. The papers are largely a record of clinical experience and since biochemical findings in the treated patients were never completely or seldom even adequately reported, there must be speculation as to the mechanisms of the reported improvement. Cliffton[145] and Ambrus[98] have reviewed the work of their respective groups in this field.

Recently a preparation of human fibrinolysin* has become commercially available, and clinical experiences with it recorded.[145-155] Since details of its biochemical characterization have not been published and the potency of the material is expressed in undefined fibrinolytic units, we have analyzed samples in our laboratory. Each vial of a stated potency of 50,000 fibrinolytic units contain 5.1 casein units of plasmin and 6,500 Christensen units of streptokinase. It is our view that the material would be more correctly described as streptokinase-activated plasmin rather than as human fibrinolysin. Unless the composition of Actase varies widely, the failure of Moser[143] and the manufacturer[156] to detect the presence of streptokinase in the material is surprising, since in our samples the streptokinase content would clearly represent the major part of any fibrinolytic moiety assayed by a fibrinolytic technique.

The knowledge that 50,000 units of Actase (the recommended dose is 50,000 to 150,000 units) contains a minute quantity of plasmin (5.1 casein units)† and a small quantity of streptokinase (6,500 units) suggests that it will cause little or no biochem-

*See previous footnote on plasmin.

*Actase. The use of the term human fibrinolysin in lieu of human plasmin can be regarded only as unfortunate. Originally fibrinolysin referred to the streptococcal principle now named streptokinase. Later the terms profibrinolysin and fibrinolysin were used synonymously with plasminogen and plasmin. This terminology has been generally abandoned chiefly because of the unjustified connotation of specificity implicit in its use but also because of its ambiguity. (See section on mechanisms of physiologic and therapeutic thrombolysis.)

†One milliliter of normal plasma contains approximately 3.5 casein units of available plasmin.

ical change on its infusion in the human.* The unsatisfactory nature of the evidence[143,146] purporting to demonstrate that important biochemical changes are produced on Actase infusion is confirmatory of this hypothesis.† It is significant that "fibrinolytic activity" has been demonstrated not during the infusion of Actase but rather one or more hours after the termination of the infusion. At this time plasminogen activator release into the circulation, secondary to the pyrogenic nature of the material,[142,146,157] might be expected to occur.[116,158,159]

Plasminogen activators. The only plasminogen activator that has received extensive clinical trial has been streptokinase. Tillett, Johnson, and McCarty[123] were the first to show that the intravenous infusion of streptokinase would produce a short-lived fibrinolytic effect in some patients. The investigation was, through necessity, exploratory, as the streptokinase available to these investigators produced pyrogenic and hypotensive responses. The development of streptokinase purification methods[124] led to a great reduction in clinical toxicity[118,125-131] and the later work with streptokinase has been with material virtually devoid of toxicity.

The biochemical basis for therapy with massive and prolonged streptokinase therapy has been fully studied.[35,95,118] It has been demonstrated that plasma thrombolytic activity can be increased several hundred-fold over resting levels in the adult not under stress and that this biochemical state can be maintained through appropriate dose adjustment for as long as is desired.[95] The sole toxic effect of the treatment, a coagulation defect, sometimes leading to a hemorrhagic diathesis, is not a bar to treatment provided that the initial condition of the patient's coagulation mechanism is satisfactory. Moreover, the administration of steroids diminishes the degree of this anomaly to within clinically tolerable bounds. The safety of the treatment should be still further increased after more experience has been gained with the intravenous use of epsilon aminocaproic acid,[92,159,160] an inhibitor of plasminogen activation. Though certain problems of an immunologic character still remain to be investigated (see earlier section), this treatment has been tested in a series of 50 patients suffering from various forms of thromboembolic disease, including 22 patients with acute myocardial infarction of short duration.[130] The results suggested that in vivo thrombolysis had been achieved and that certain theoretical objections that could have been relevant to the use of such therapy in myocardial infarction were without practical basis.

Recently Johnson and McCarty[131] have studied the effect of intravenous streptokinase infusion on experimentally induced thrombophlebitis in the human. Volunteers submitted to limited vein trauma and, as a consequence, developed a firm clot within a small segment of venous lumen. Control observations showed no tendency toward spontaneous resolution and untreated lesions underwent organization. Patients were treated 24 to 48 hours after the production of the venous lesion with streptokinase infused intravenously into the contralateral limb. In a high proportion of treated patients, complete resolution of the venous lesion resulted. Though the authors' laboratory methods and biochemical interpretations are controversial, this demonstration of the in vivo thrombolytic effect of streptokinase infusion in the human is of great importance.

Other drugs inducing in vivo plasminogen activation. Though not strictly within the scope of this review, mention will be made of materials which on injection cause in vivo plasminogen activation through in-

*In man total circulating plasma antiplasmin values run between 10,000 and 20,000 casein units[95] and total circulating plasma streptokinase antibody 6×10^3 to 6×10^6 units, depending on the patient's state of immunization.[133]

†Limited personal experience (6 patients) with Actase infusion (50,000 to 100,000 fibrinolytic units) with the use of extremely sensitive assay techniques[95,117] has failed to reveal any significant biochemical changes in plasminogen system components either during or for 2 hours after the infusion.

direct and ill-understood mechanisms. This aspect of the subject may bear promise for the development of a new type of thrombolytic therapy in man. Stress situations such as therapeutic electroshock, exercise, or ischemia, and miscellaneous drugs such as epinephrine, acetylcholine, and nicotinic acid are known to cause a release of plasminogen activator into the circulation.[116, 161-170] With all these procedures plasminogen activator release is transient. The recent studies of Kwaan, Lo, and McFadzean[163-167] appear relevant to the possible mechanisms involved. Though at present efforts are being made to develop nicotinic acid as a therapeutic tool for releasing activator, clinical trial has not been attempted since the effect is variable, short lived, and not reproducible (tolerance develops rapidly).[171]

On the other hand, the injection of a pyrogen causes, after a latent period and often before the onset of the temperature spike, a more sustained rise of plasminogen activator concentration. This phenomenon has been extensively studied,[116,158,159,172-177] a bacterial pyrogen* being chiefly employed. Because of side effects and the rapid development of tolerance, however, definitive clinical trial has not been reported.

Interpretative therapeutic evaluation. Despite the great promise inherent in the concept of in vivo thrombolysis produced by the intravenous infusion of plasminogen activators, much remains to be learned and proved. The findings discussed have all been obtained at a clinical investigative level and further knowledge must be acquired before standard treatment schedules can be properly tested.

Progress to date has been in biochemical and physiologic areas and therapeutic effect has in fact been incidental to the acquiring of fundamental knowledge. The reviewers would emphasize that in their view the numerous clinical trials conducted in this area without adequate biochemical and physiologic measurement have contributed nothing but confusion. It is irrational to believe that the principles of this therapy can be ascertained by hit and miss trial in the human. The only sound experimental approach is to determine the various biochemical approaches made possible by the possession of various agents and then to test the clinical efficiency of controlled biochemical states in various disease situations.

V. Conclusion

The use of proteolytic enzymes as therapeutic agents has grown rapidly in recent years. Although a considerable and most enthusiastic literature on these subjects has accumulated, evaluation of most of the reports has been hampered by several general considerations: in some areas, certain agents have been used whose rationale depends on biochemical observations in purified systems alone but where a similar rationale in complex biologic systems does not exist; in other areas, empirical observations dominate the use of these enzymes, yet little scientific data support their use; and, in still others, uncontrolled clinical observations have been made on the effects of these agents in diseases where the natural course is extremely variable or where the effect of other agents, simultaneously administered, has not been clearly defined. Unfortunately, practical therapeutic considerations have so dominated this area of pharmacology that the needs and requirements for proper development often have been overlooked or bypassed in the rush to apply these enzymes clinically. The confusion which has resulted only serves to emphasize the real needs in this field, i.e., correlative basic and clinical study with a view toward developing effective therapeutic agents, rather than the appraisal of agents prematurely released for clinical use.

Initially applied directly to diseased areas for local action, enzyme therapy now is being extended on a systemic basis to bring under attack undesirable materials accumulating extracellularly in various parts of

*Pyrexal.

the vascular bed or connective tissues of the body. This concept of systemic therapy with enzymes considerably extends the potential usefulness of these agents for therapeutic purposes; achievement of this goal with practical and effective methods would significantly alter the course of many diseases prominent in morbidity and mortality statistics.

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Orally active hypoglycemic substances and the rationale of their use

The introduction of the orally active hypoglycemic agents has already had a profound effect on the therapy of diabetes. Unfortunately, despite a great deal of discussion in the literature, there is still much about these drugs which is uncertain and unsettled and, in general, information about their limitations is not so widely spread as claims for their utility. Although the modern era of interest in oral hypoglycemic agents began about 40 years ago with the association of this effect with the guanidines, it was first observed and confirmed more than 80 years ago as the result of salicylate therapy. Hypoglycemic effects have been noted after the oral administration of such unrelated materials as (1) sulfonamides and sulfonylureas (e.g., tolbutamide), (2) guanidines (e.g., synathalin A), (3) extracts of the akee plant (hypoglycin A and B), and (4) salicylates. It is also reasonable to suppose that more are on their way. The state of our knowledge of the modes of action of the several groups of orally active hypoglycemic agents based on clinical and experimental observations as well as their clinical uses, limitations, and dangers are discussed.

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The history of the orally active hypoglycemic substances begins with the observations of Watanabe[1] in 1918 that guanidine lowers the blood sugar of animals. Eight years later Frank and his associates[2] introduced an oral antidiabetes preparation, synthalin, which is the decamethylene derivative of guanidine. Because of local irritating effects in the gastrointestinal tract and the suspicion of liver damage, the clinical trials of this material were short lived. In 1929 a series of biguanides were synthesized[3] and tested on animals for their hypoglycemic activities.[4] Because of the exhibition of severe toxicity these compounds were not tested clinically at that time. During the past 3 years interest in this chemical group has been reawakened and much active experimental and clinical work is proceeding in this area (see below).

In 1942 Janbon and co-workers[5] were using a new sufonamide drug, synthesized by Vonkennel and Kimmig,[6] and observed the occurrence of severe hypoglycemia in their patients, especially in poorly nourished individuals. This drug was 2-(*p*-aminobenzol-sulfonamido)-5-isopropyl thiodiazole, henceforth referred to as IPTD. During the next 4 years Loubatières and his group[7] investigated this and closely

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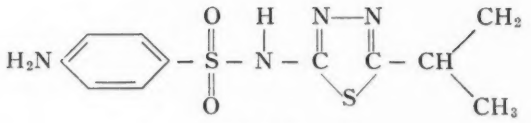

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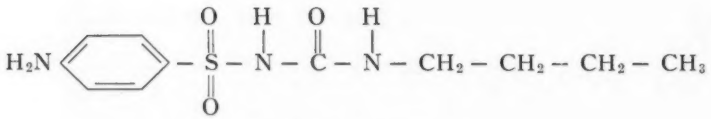


BZ 55 (Carbutamide)

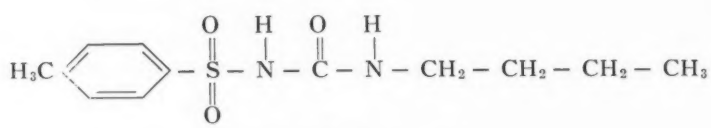
related compounds in animals. They succeeded in establishing the basic pharmacologic actions of these drugs and proposed the theory that they lower blood sugar by releasing insulin from the beta cells of the pancreas. No clinical trials were undertaken with IPTD until about 1955.[8] The early work of Loubatières was confirmed by La Barre and Reuss[9] in Belgium and by Chen[10] in this country. Again no clinical trials were instituted. In 1955 there appeared a series of papers on the synthesis,[11] hypoglycemic activity,[12] pharmacology,[13] and clinical application[14] of another sulfonamide derivative, the butyl-urea of para-amino sulfanilamide, known as BZ 55.

Because of the relative freedom from toxicity in man and animals of the first sulfonylurea tested, feverish chemical and pharmacologic activity ensued. It was very soon realized that one could dispense with the para-amino group on the benzene ring of the molecule without significant loss of hypoglycemic activity, and gain the advantage that such a molecule would no longer possess antibacterial potency. Thus the compound D 860 or tolbutamide was released for testing early in 1956.[15] The extent of activity in the field of hypoglycemic sulfonamide derivatives may be judged by the fact that the two major firms in this field have made over 700 compounds and tested many of them in the space of 3 to 4 years. The newest member of the group now in general use is the isopropyl chlor-substituted derivative, chlorpropamide.[16]

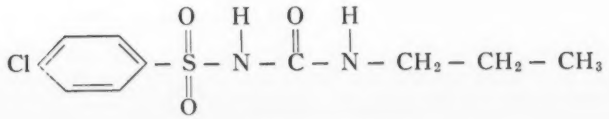
In addition to the sulfonylureas and biguanides this paper will also consider briefly the hypoglycemic amino acid derived from the akee plant,[17] and the use of salicylates in diabetes. The latter is a revival of interest in an action first reported in 1876.[18]

The sulfonylureas and related substances

The study of the relation of structure to pharmocologic activity is of importance both academically and practically. We wish to understand mechanism of action on a molecular level, if possible; and therefore the "active" groupings of a drug are sought



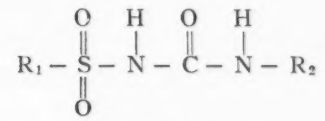
D 860 (Tolbutamide)



Chlorpropamide

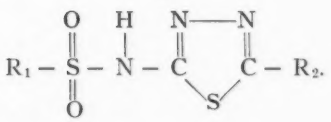
for. We also should like to fashion a drug for use in a chronic disorder (like diabetes) in such a manner that the maximum possible activity is coupled with the barest minimum of toxicity.

Ruschig and co-workers[19] have recently published a systematic examination of this problem, with an extensive compilation of the essential aspects of the chemistry of the hypoglycemic sulfone derivatives. This and related studies may be summarized as follows: The necessary core or center grouping in the molecule seems to be either



Sulfonyl urea

or



Sulfathiodiazole

The front (R_1) and hind (R_2) ends of the molecule may be varied rather extensively. Such varied substituents determine the degree of hypoglycemic activity, its duration, the extent of toxic side effects, etc. In the commonly used members of the group R_1 is a substituted benzene ring—para-aminobenzol in IPTD and carbutamide, methylbenzene in tolbutamide, and chlorobenzene in chlorpropamide. R_2 is either a butyl or propyl group. The central portion is (-SO_2-urea). At present the only -sulfonylthiodiazole under investigation is IPTD in which R_1 is para-aminobenzene and R_2 is an isopropyl group.

Those drugs possessing the para-amino group have antibacterial potency, which in itself is not a desirable quality for long-term use. In addition, perhaps by reason of such a group, side reactions especially of the "allergic" type seem to be a significant feature of their over-all effects. When the substituent on the benzene ring is CH_3 or Cl, no antibacterial effects are obtained and the degree of toxicity of therapeutic doses is considerably lessened. Chlorpropamide has greater hypoglycemic activity per unit weight than does tolbutamide. This has been termed "greater potency," legally a correct statement. The phrase as used in advertisements has however acquired an undue emotional connotation. Since tolbutamide is less active per unit weight than chlorpropamide, it has naturally led to fewer "hypoglycemic" episodes in clinical practice. No scientific basis is however available for calling the drug "euglycemic"—whatever that may mean.

While the physiologic basis of the blood sugar–lowering effect is partially understood there is as yet no hint as to the relation of chemical structure to the basic biochemical events in the cell which constitute the primary molecular action of these drugs.

The hypoglycemic sulfonamides

To define fully the relationship of these agents to the diabetic state it seems necessary to distinguish between the following properties: (1) the ability to lower the blood sugar in the patient with established diabetes; (2) the ability to increase the resistance of an animal to the development of the diabetic state on exposure to an adequate diabetogenic stimulus, i.e., "antimetadiabetes." Both properties have been demonstrated by the hypoglycemic sulfonamides. The latter may have important prophylactic significance, separate from its usefulness in therapy. Each will be discussed separately. Loubatières[20] has emphasized the importance of this distinction.

The hypoglycemic effect. To understand the mode of action of any agent which lowers the blood sugar the following questions must be answered:

1. Does it exert its action on the extrahepatic tissues to promote glucose uptake directly?

2. Does it exert a direct action on the liver to reduce liver sugar output?

3. Does it reduce the activity and/or output of the "diabetogenic hormones" (primarily the anterior pituitary and adrenal

cortical secretions as well as glucagon and/or epinephrine)?

4. Does it interfere with the catabolism (? insulinase) of the insulin molecule and thereby enable very small amounts of insulin to exert a major effect?

5. Does it activate the beta cell to produce and/or release extra insulin into the circulation ("beta cytotropism")?

As Loubatières has pointed out,[20] the majority of pharmacologic substances are not characterized by a simple, specific, exclusive mechanism of action. In addition to a principal action there probably exist secondary ones which, depending upon circumstances, may become more prominent than the principal action. The hypoglycemic sulfonamides are not exceptions to this rule.

1. Direct action on the extrahepatic tissues. Two experimental situations have been used to evaluate a possible direct action on the extrahepatic tissues: in vitro incubation of tissues; and the eviscerated animal.

Studies on the effect of carbutamide and tolbutamide added in vitro on the glucose uptake of the isolated rat diaphragm have yielded some discordant results. A majority of investigators have failed to show any effect[21-27] but some have reported increased glucose uptake by this muscle preparation.[28-30] Rafaelsen[30] has attempted to understand these discrepancies by emphasizing the following points in methodology which he regards as particularly relevant: the duration of the incubation period (since the rate of glucose uptake falls with time,[31] the shorter the incubation period the greater will be the glucose uptake per unit of time); the nature of the buffer (it is easier to demonstrate glucose uptake in bicarbonate-buffered than in phosphate-buffered media[32,33]); the sensitivity of the test system as measured by the smallest concentration of sulfonamide in the medium (he could show a significant effect with carbutamide in concentrations between 10 and 70 mg. per 100 ml., but not with lower or higher concentrations).

Recently Renold and co-workers[34] have studied in vitro the effects of tolbutamide on the metabolism of uniformly labeled C^{14} glucose by rat adipose tissue. They found increased production of CO_2 and a reduced rate of lipogenesis. Since increased oxidation of glucose is usually accompanied by a parallel increase in fatty acid synthesis from glucose carbon the experiments were repeated with glucose-1-C^{14} and glucose-6-C^{14} as substrates. It was then found that the major source of the newly generated CO_2 was carbon-1 of the glucose molecule, suggesting increased activity of the phosphogluconate oxidative pathway. Again lipogenesis was impaired. Chlorpropamide gave the same results. These data were taken to suggest interference with the transfer of hydrogen from glucose-6-phosphate to the fatty-acid chain, perhaps at the level of the transferring pyridine nucleotides. This interpretation is consistent with and similar to the Wallenfels[35] hypothesis of the mechanism of action of the sulfonylureas. The hypoglycemic sulfonamides have been uniformly ineffective in eviscerate preparations; the dog,[36,37] rabbit,[38] and rat[39,40] have been studied.

Other work relevant to the problem of peripheral action must be mentioned. The laboratories of Houssay[41] and Loubatières[42] have reported a potentiating effect of the hypoglycemic sulfonamides on exogenous insulin when given to the normal or pancreatectomized dog. These experiments will be discussed below; although they admit of several possible interpretations, a direct peripheral effect is possible.

The question as to whether these agents affect the metabolism of the extrahepatic tissues has been recently considered in summary form.[43] Although final judgment must be reserved until further evidence is available, to attribute a major portion of the hypoglycemic effect of these agents in humans in the doses which are clinically useful to a direct tissue effect would be contrary to the bulk of present evidence.

2. Direct action on the liver. There is now much evidence which has been interpreted

to mean that one of the effects of the hypoglycemic sulfonamides in the intact organism is suppression of liver sugar output. Since this may be viewed either as a direct action or as an effect secondary to the liberation of endogenous insulin into the portal vein, we shall defer consideration of experiments with the intact animal to a later section and concern ourselves in this section with work dealing with the direct hepatic effect.

If the beta cells are intact, sulfonylureas lower the blood sugar of the hepatectomized animal.[40,44] The liver is thus not necessary to the hypoglycemic effect of these agents. When liver slices are incubated with sulfonylureas, measurement of the amount of glucose released when compared with control amounts has given contradictory results. Clarke[45] was able to show an inhibitory effect of chlorpropamide when he used liver slices from normally fed rats and concentrations of the drug equivalent to therapeutic levels. Vaughan[46] could show no effect of tolbutamide on the release of glucose from rat or rabbit liver slices at concentrations greater than therapeutic levels. She did find, however, that the increment of glucose release produced by glucagon and epinephrine was significantly reduced by tolbutamide. To distinguish whether this inhibition was at or below the phosphorylase level (conceivably there might have been inhibition of phosphoglucomutase or glucose-6-phosphatase insufficient to alter the control rate of glucose release but sufficient to make one of these reactions rate limiting when the phosphorylase step was accelerated) the effect of tolbutamide on the glucose output was studied after the addition of glucose-1-phosphate to the medium. There was no inhibition. It was therefore proposed[46,47] that sulfonylureas inhibit phosphokinase, the enzyme which catalyzes the formation of active phosphorylase. Berthet and associates[48] have reported that tolbutamide and carbutamide inhibit phosphorylase reactivation in a liver homogenate system. However, extremely high concentrations of the drugs were used, and with several sulfonyl compounds tested there was no correlation between this in vitro effect and the in vivo hypoglycemic potency.

It is generally felt that the sulfonylureas do not cause significant inhibition of liver glucose-6-phosphatase activity.[46-49] The reported reduction in activity of this enzyme in rat liver after several days of treatment[49,50] follows the development of hypoglycemia and may not represent a direct effect of the drug.

It has been shown clinically that the administration of adrenaline[51] or glucagon[51,52,53] to nondiabetic and diabetic subjects induced expected normal hyperglycemic responses despite plentiful amounts of sulfonylureas. This can be reconciled with the argument from in vitro demonstrations suggesting inhibition at the phosphorylase level, if one considers significant the fact that the in vitro demonstrations required drug levels several times as high as those which lower the blood sugar clinically.

Renold and associates[54] did comparative glucose, fructose, and galactose tolerance tests in 4 patients treated with sulfonylureas in an effort to evaluate hepatic glucogenesis. There was no alteration in glucose, fructose, and galactose tolerance but there was a reduction in the increment in blood glucose which normally follows the administration of fructose and galactose to diabetic subjects. This diminished conversion of both fructose and galactose to glucose suggests inhibition of the reaction catalyzed by glucose-6-phosphatase since this is the only reaction common to the conversion of both galactose and fructose to glucose. However, an increased disposal of produced glucose into the periphery would yield similar results. Renold and co-workers[34] have also shown that tolbutamide significantly inhibits the formation of ketones by rat liver slices in vitro at therapeutic levels whereas insulin was not effective in the same system. Other hepatic enzymes have been studied.[55,56]

The intraportal infusion of carbutamide

failed to lower the blood sugar in dogs; a similar amount injected into the pancreatic artery led to hypoglycemia.[57]

3. Reduced activity and/or output of the "diabetogenic hormones." Lowering of the blood sugar could reflect interference with the release or activity of secretions from the anterior pituitary, the adrenal cortex, the thyroid, the adrenal medulla, or possibly the alpha cells of the pancreatic islets (the presumed site of glucagon production). The importance of these structures to the action of the hypoglycemic sulfonamides can be assessed if one considers experiments on the animal deprived of these glands, and evidence for altered pituitary, adrenal cortical, and/or thyroid function in the treated subject.

The endocrine glands other than the pancreas are not necessary to the demonstration of sulfonamide hypoglycemia. Loubatières[20] has shown this in the dog lacking the pituitary, adrenal, thyroid, and parathyroid glands, and the gonads. Houssay and Penhos[37] have shown that these agents lower the blood sugar in hypophysectomized as well as adrenalectomized animals. This effect was similar in degree in hypophysectomized and normal dogs, but the effect was more prolonged and attended by greater toxicity in hypophysectomized rats than in the normal control group. The adrenalectomized animals of three species (dogs, rats, and toads) exhibited marked sensitivity to the hypoglycemic and toxic actions of the drugs. LaBarre and Reuss[9] found that the IPTD effect was much more intense and prolonged in the adrenalectomized dog when compared with the control animal; and Lang and Sherry[39] showed that rats with complete removal of the adrenal glands or of the adrenal medulla are more sensitive to tolbutamide. Thus it is seen that removal of the anterior pituitary and/or its various target glands does not inhibit the hypoglycemic effect of the sulfonamides, but in fact increases it in many instances.

There is no significant or consistent depression in the urinary excretion of 17-hydroxy- or 17-ketosteroids in the treated patient.[53,58-60] Fajans and co-workers[58] could find no metabolic changes suggesting decreased adrenal cortical activity. In one patient with coexistent diabetes mellitus and Addison's disease, tolbutamide reduced the blood sugar despite steroid replacement therapy; steroid withdrawal did not potentiate the hypoglycemic effect. There was no evidence to suggest interference with the peripheral effects of the adrenal corticoids. Neither the diabetogenic nor the other metabolic effects of prednisolone were altered in the treated subject.

Fajans[58] found no alteration in the mild diabetes of one patient with active acromegaly given tolbutamide. Bergenstal and others[59] were able to control 2 cases of acromegaly with 2.0 Gm. of tolbutamide daily. Cardonnet and associates[61] found no suppression of urinary gonadotropins after 3 months of chlorpropamide therapy.

These agents appear to have a small but definite inhibitory effect on thyroid function. They induce thyromegaly in the rat when administered in the diet[13,62] and thus share this goitrogenic property with other sulfonamides.[63] Carbutamide and tolbutamide in therapeutic doses have an antithyroid effect in some human subjects, carbutamide being more active in this respect.[64,65] The mechanism of the antithyroid effect of carbutamide does not seem to be thiocyanate-like. Chlorpropamide appears to have minimal influence on thyroid function. Cardonnet[61] could show no significant effects after 3 months of treatment. Hamwi and co-workers[60] found slight depression of thyroid function but no clinical manifestations of thyroid deficiency or goiter.

THE ALPHA CELLS OF THE PANCREATIC ISLETS. Although glucagon has not been shown to exert an anti-insulin effect under all circumstances and the hypoglycemic sulfonamides are ineffective in severe alloxan diabetes,[7] when the *alpha* cells are preserved, a group of German workers[12,14,66,67] suggested a destructive action of these drugs on the alpha cells as the mechanism of hypoglycemia. They supported their

views with histologic evidence. Other workers[52,68] could find no histologic change in the alpha cells. More recent publications[69,70] by the same groups which originally spoke of alpha cell inhibition now question the validity of the hypothesis.

In vivo the drugs do not significantly alter the hyperglycemic action of glucagon.[48,51-53,68] In vitro experiments, however, with liver and very high drug levels, did suggest inhibition of glucagon action.[48]

4. Interference with the catabolism of the insulin molecule. To decide whether the hypoglycemic sulfonamides interfere with the catabolism of the insulin molecule the following experiments seem to be particularly relevant: those attempting to demonstrate inhibition of insulinase in vitro; those designed to demonstrate a potentiating effect on exogenously administered insulin; those which define the rate of degradation of I^{131}-labeled insulin in vivo; and studies on the insulin sensitivity of the sulfonamide-treated subject.

Mirsky and collaborators[71-73] have presented evidence that the sulfonylureas lower the insulinase content of livers, when given in vivo; and that they inhibit the action of insulinase in liver homogenates (but not in more highly purified preparations). On the other hand Vaughan[47] has shown that the concentration of sulfonamide necessary for the production of hypoglycemia in the rat was much less than that required to reduce the degradation of insulin by a liver enzyme preparation in vitro.

A potentiating effect on exogenous insulin has been shown in the normal and in the pancreatectomized dog.[41,42,74] This interesting property has been claimed by Loubatières[75] after the administration of very large doses of insulin (10 units per kilogram of body weight) by *mouth* to the normal or pancreatectomized dog. The disappearance rate of I^{131}-labeled insulin from the blood is apparently not altered by the sulfonylureas.[76] Neither in the animal[39] nor in the human[58] has there been produced a significantly altered sensitivity to exogenous insulin.

5. Activation of the beta cells. There can be little question at this time that the integrity of the beta cell is fundamental to the action of the hypoglycemic sulfonamides. These agents are active in several animal species (monkey, dog, cat, guinea pig, rat, mouse, and toad) when the pancreas is intact[8,37,41,42,77]; they are inactive if total pancreatectomy is done. This has been shown in the case of the dog,[8,36,37,78-81] the cat,[82] and the toad.[37] If as little as one-twentieth of the initial weight of the gland is left in the abdomen, however, the drugs may reduce the blood sugar level.[8] In the hypophysectomized pancreatectomized dog, which is sensitized to the development of hypoglycemia, these agents do not acutely lower the blood sugar.[83] In the animal with severe alloxan diabetes, where selective injury to the beta cells is the presumed cause, these drugs do not lower the blood sugar.[8,20] In the chicken and the duck the pancreas is not necessary for the hypoglycemic action of the sulfonylureas.[84]

These experiments are consistent with observations in the human. Whereas the drugs are active in normal man they are inactive in the pancreatectomized human being[58,85-87] unless the pancreatectomy is incomplete.[88] Where the diabetes in the human is on the basis of hemochromatosis (the pancreatic hemosiderin deposition characteristic of the condition restricts itself to the beta cell[89,90]) these agents are inactive.[91,92,93] The juvenile patient with established diabetes, generally conceded to have little or no beta cell function, is not likely to be benefited by these drugs.[14,94-96]

The many in vivo observations compel one to accept the conclusion that the hypoglycemic sulfonamides somehow promote the release of additional insulin from the beta cells of the islets of Langerhans, a phenomenon spoken of as "beta cytotrophism." It is fair to ask whether increased amounts of an insulin-like material can be found in pancreatic venous blood following the administration of these agents. Increased "plasma insulin activity" has been shown by in vitro assay methods with the rat hemi-

diaphragm[97-99] or the epididymal fat pad[100]; and by in vivo experiments involving cross-circulation techniques (such as pancreaticoduodenal-jugular anastomosis).[7,101,102]

Final support for the betacytotrophic hypothesis comes from histologic work which has shown that acute administration of the drugs causes degranulation of the beta cells.[103-106] The important microscopic changes seen in the chronically treated animal are considered in a later section. Thus, largely from work on the pancreatectomized or alloxan diabetic animal, various pancreatic venous assay efforts, and histologic studies, the betacytotrophic hypothesis has won general acceptance. The question of the mechanism of this betacytotrophism, neural or direct, next suggests itself.

A series of experiments by Loubatières and associates[7,20,77,107] directed toward this problem tends to exclude a neural mechanism. When in the dog destruction of the brain from the frontal lobes to the posterior limit of the corpora quadrigemina was followed, in succession, by destruction of the midbrain, removal of the pituitary, division of both vagi in the neck, and bilateral ligation of the carotid arteries, "sulfonamide" hypoglycemia was still demonstrable. These workers (unpublished experiments) were also able to show sulfonamide activity in dogs with a pancreatic graft under the skin of the abdomen (connected to the circulatory system by its vascular pedicle).

Arguing for a direct chemical effect by the agents themselves are the experiments which report that the injection of very small amounts of a sulfonylurea directly into the pancreatic artery results in hypoglycemia.[7,57,77] We have suggested that the hypoglycemic sulfonamides, by a direct mechanism, stimulate the beta cells of the islets to release a material into the pancreatic venous effluent which behaves like insulin. The newly liberated material reaches first the liver and then the extrahepatic tissues. A central problem in insulin action concerns the nature of this hepatic transit of the insulin molecule; does it exert a direct action on the liver to reduce liver sugar output and thus contribute to hypoglycemia by a hepatic mechanism? The insulin which survives this hepatic passage (and not all of it does, since the plasma insulin level in a peripheral vessel is not as high as that in the pancreatic vein following sulfonylureas[100]) is of course free to act on muscle, adipose tissue, and other insulin-responsive tissues.

Consideration of the evidence for and against an action of insulin on the liver is beyond the scope of the present communication. A recent symposium has considered this question.[108]

"Antimetadiabetic" effect. The hypoglycemic sulfonamides have been shown to oppose the development of the diabetic state on exposure to an adequate diabetogenic stimulus. Working with the animal with alloxan metadiabetes Loubatières[20,80,108] has shown that if the diabetes is of moderate intensity the institution of sulfonamide therapy generally results in "cure" in 3 to 8 weeks, i.e., treatment may be discontinued and diabetes does not return. The earlier the treatment is instituted and the less intense the diabetes the more effective are the sulfonamides. Similar studies demonstrating sulfonamide opposition to hypophyseal metadiabetes have been made[107,109,110] and a "cure" has been reported in a spontaneously diabetic dog.[111]

It has been shown in the rat and rabbit that administration of the hypoglycemic sulfonamides results in the formation of new beta cells after several days.[20,103,104,112] Two mechanisms appear to be involved in the genesis of these new cells:

1. Mitosis of the existing beta cells (this was demonstrated by the colchicine technique).[113]

2. Metaplasia of the various cells constituting the exocrine portion of the pancreas (acini and ducts), i.e., *"exo-endocrine transformation."*[103,113] Loubatières[114] has been able to identify forms intermediate between acinar and beta cells. The new beta cells form "microislets."

Can this ability to stimulate the generation of new beta cells explain the resistance

of the treated animal to metadiabetes? It has been shown that in moderately severe meta-alloxan diabetes some beta cells are spared[20,79,80,115] and Loubatières has shown the formation of new beta cells in his dogs "cured" of alloxan metadiabetes. He has found that these newly generated cells are able to respond normally to alterations in the blood glucose level and also respond acutely to the hypoglycemic sulfonamides. Finally, they are destroyed by alloxan since alloxan has induced diabetes in animals previously diabetic but "cured" by the sulfonamides.[114]

Metahypophyseal diabetes has been generally attributed to exhaustion, degeneration, and destruction of the beta cells.[109,110] It would be fair at this time to attribute the resistance to metadiabetes encountered on sulfonylureas to the formation of new beta cells.

The sulfa derivatives which produce hypoglycemia seem to have a uniform action as a group. Despite the prodigious amount of work on the subject it is not yet possible to get a detailed, clear picture of the mode of their actions.

It is evident that the liberation of insulin via a direct effect on the beta cell is the principal and probably initial point of attack. The insulin-potentiating effect in the depancreatized animal which can be elicited under certain conditions may, on a molecular level, be very similar to the beta cell effect. Both may depend upon freeing insulin from a complex. In the absence of the hormone hypoglycemia is not obtained in acute experiments. These and other considerations previously discussed demonstrate the lack of any significant direct effect upon tissues (including liver) in respect to the metabolism of sugar. Absence of the liver provided the pancreas or insulin is present does not prevent the hypoglycemic effect. It is unclear how much of a role is played by a possible insulinase inhibition.

If the predominant action is, then, provision of insulin, one should expect that following administration of the hypoglycemic sulfonamides one should find all the metabolic consequences of an insulin injection. Such comparative experiments have been done in many ways. The results are by no means uniform with respect to peripheral glucose utilization,[26,116-120] phosphate and pyruvate changes,[116-118] respiratory quotient,[7,121] etc.[122-124]

A synthesis of these divergent results has been proposed by Madison and co-workers[125,126] and by Weinhouse's[127] laboratory on the basis that ordinarily the sulfonylureas liberate insulin into the portal system. The hormone reaches the liver first, acts there to inhibit sugar output or increase its uptake and is retained in that organ to a large extent. Only when an "excess" of insulin is present does a portion reach the peripheral circulation and act in the extrahepatic tissues. This view and the data published by these workers go a long way toward reconciling divergent results. Complete harmony does not as yet exist, since it is not accepted by all workers that both insulin and tolbutamide inhibit liver sugar output.[128-130] The mode of action of the hypoglycemic sulfonamides will not become clear at least until the controversy concerning the nature of the hepatic effects of insulin is settled.

The guanidine family

A hypoglycemic effect of phenethylbiguanide (PEBG), a water-soluble condensed diguanidine, was demonstrated by Ungar and collaborators[131] in the monkey, guinea pig, rat, rabbit, and cat. The maximal fall in blood sugar occurs 5 hours after oral administration. This agent is similar to chloroguanide hydrochloride* (chemical structure), a biguanide which has long been used in the treatment of malaria without showing hypoglycemia,[132] and synthalin A,[2] a hypoglycemic guanidine withdrawn from clinical use some 30 years age because of presumed liver toxicity.

PEBG does not lower the blood sugar of normal human subjects[133] but it is effective in diabetes, both adult and juvenile

*Paludrine.

types.[134-138] Wick and Stewart[139] working with C^{14}-labeled PEBG, recovered 90 per cent of an administered dose in the urine within 24 hours. Prior to its excretion the drug was concentrated almost exclusively in the liver and the gastrointestinal tract.

Glucose uptake by the extrahepatic tissues. Classically designed experiments directed toward the question of an action on the extrahepatic tissues strongly support such an effect. PEBG has been incubated in vitro with the hemidiaphragm or the epididymal fat pad. Its effects on the diaphragm include: acceleration of glucose uptake,[131,140,141] decrease in oxygen consumption,[141] increase in lactate production,[140] reduction in glycogen content,[140,141] inhibition of the enzyme which catalyzes the conversion of inactive to active phosphorylase,[141] and no action on pentose transport.[142]

Wick, Larson, and Seuf[143] have shown the following effects of PEBG on the fat pad: reduced oxygen consumption, inhibition of the oxidation of glucose, acetate, and succinate, and inhibition of fat synthesis.

The in vitro demonstration of increased glucose uptake by the diaphragm has been confirmed by Nielsen and associates[144] in the eviscerated guinea pig and Butterfield and co-workers[120] in the isolated limb. Work on the intact animal further supports the in vitro data. Williams and his collaborators[141] could show no increase in CO_2 or $C^{14}O_2$ production after the administration of C^{14}-labeled glucose and there was no increased incorporation of C^{14} into the protein or lipid fractions of diaphragm, adipose tissue, and liver slices removed from PEBG-injected animals and incubated with C^{14}-labeled glucose. In animals increased blood lactate levels—preceding hypoglycemia—have been shown.[140,145] In diabetic man increased blood lactate and pyruvate levels have been shown in the fasting state and during a glucose tolerance test[133] and slow disappearance during a pyruvate tolerance test.[146] A decrease in muscle glycogen has been shown[141]; the respiratory quotient is increased[147]; and the inorganic phosphorus level is increased.[145]

PEBG then, like insulin, seems to promote glucose uptake by the extrahepatic tissues and increase lactate and pyruvate production. The similarity soon ends, however. First, the failure of PEBG to expand the pentose space of the isolated rat diaphragm as insulin does would argue against an action at the cell surface. Second, the glucose which gains entry to the cells as an expression of PEBG effect is not available for glycogenesis, lipogenesis, or oxidation. The following triad of effects—increased glucose uptake, diminished oxygen consumption, and increased production of lactic acid—may be presumed to represent accelerated anaerobic glycolysis secondary to an inhibition of oxidative metabolism with loss of the Pasteur effect. This hypothesis receives support from work demonstrating a PEBG inhibition at the cytochrome level[148] and on the succinic oxidase system.[143]

At this point in the argument one meets some difficulty in applying these experiments to the clinical situation. If the mode of action of PEBG were indeed to inhibit oxidative metabolism, serious toxicity would be expected. However, a fairly extensive clinical experience with the agent does not support this expectation. One must therefore choose between two possibilities: (1) the true in vivo action does not represent inhibition of oxidative metabolism and its consequences; (2) the mode of action is similar to that suggested by the above work but for some unknown reason serious toxicity has not yet appeared.

One cannot resolve the problem at present.

Suppression of liver sugar output. As was the experience with the rat diaphragm, adding PEBG to a liver slice causes an increase in the glucose uptake,[140] an increase in lactate production,[140] a decrease in oxygen consumption,[140,145] and a decrease in its glycogen content.[140] Studies on liver mitochondria have shown inhibition at the levels of succinate oxidation[143,145] and the cytochromes.[145] In contrast to this effect on glucose uptake there appears to be no inhibi-

tion of glucose-6-phosphatase activity[141] and no effect on liver glucose output.[140]

In vivo work has given contradictory results. PEBG will produce hypoglycemia in the hepatectomized animal maintained on glucose[144] and thus the liver is not necessary to its action. Tranquada, Kleeman, and Brown[149] studied the effect of PEBG in 5 human diabetic patients after right hepatic vein and femoral artery catheterization and found no reduction in liver sugar output during the period of hypoglycemia. There was a 30 per cent increase in hepatic blood flow, no change in hepatic vein urea, pyruvate, or lactate levels, and no change in liver oxygen consumption.

On the positive side there is work showing a decreased rate of glucose release from the guinea pig liver following PEBG.[144] Some[144] but not others[133] could show a fall in the hypoglycemic response to glucagon or epinephrine. A reduction in gluconeogenesis[141] and glycogenesis following alamine[141] or glucose[147] has been reported. Liver urea production has been shown to fall[140] and the liver glycogen content falls in vivo.[141] Consistent with the in vitro work no C^{14} (from C^{14} glucose) could be found in the protein or lipid fractions of liver in PEBG-treated animals.[147]

Influence on t[illegible] diabetogenic hormones. Fajans and as[illegible]s[133] could find no significant alterati[illegible]he endocrine function of normal human [illegible]ects given PEBG and Bergen[150] could [illegible] no impairment of adrenal function [illegible] diabetic patients who responded to PEBG.

Anti-insulinase action. PEBG has been shown[141] to have no significant capacity to inhibit the degradation of I^{131}-labeled insulin.

Toxic effects. In animals death from overdosage appears to be a direct result of hypoglycemia although Wick and Larson[151] have shown that high concentrations of the drug can induce cardiac arrythmias. Extensive clinical experience with PEBG[133-135,150] has shown no serious toxicity. Side effects such as nausea, vomiting, diarrhea, and metallic taste often limit its use, but there have been no known renal, hepatic, or hematopoietic damage and no fatal accidents.

Hypoglycin A and B

The syndrome usually known as the vomiting sickness of Jamaica, which includes hypoglycemia as a major feature,[152, 153] is generally felt to represent a poisoning due to the ingestion of the unripe fruit of the *Blighia sapida* tree.[154] Hassall and co-workers[155, 156, 157] first isolated the active hypoglycemic materials and these have been recently characterized by von Holt and Leppla[158] as a 7 carbon cyclic amino acid and its dipeptide with glutamic acid.

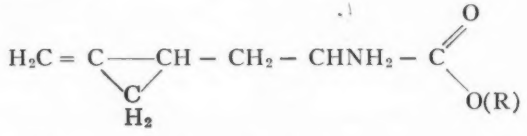
The clinical syndrome[153] has been reported only from Jamaica although the tree is also cultivated in Southern Florida and Africa.[159] It occurs sporadically or in small family outbreaks usually during the winter months from November to March when food is particularly scarce among the Jamaican peasants. The onset is abrupt and characterized by severe vomiting without diarrhea or fever; there are hypotension and tachycardia. A latent period lasting several hours sometimes follows, during which there is an illusion of improvement. In the terminal phase there are drowsiness, twitching, convulsions, and coma. Sometimes there is effortless vomiting. The mortality is high; the total course in the fatal cases lasts an average of 12 hours. Patients who recover do so completely. Occasionally a fulminating clinical variant without vomiting is seen, the patient succumbing in several hours. Children between the ages of 3 and 10 years seem particularly susceptible and the disease generally occurs in a setting of poverty and malnutrition, patients often losing weight as well as showing signs of protein and vitamin B deficiency.

Scott's[154] view that the illness is caused by ingestion in some form of the unripe fruit of the *Blighia sapida* tree has not found complete acceptance. This fruit (colloquially known as "ackee" or "akie" in Jamaica and "ishin" in Nigeria) serves as an important article of diet in Jamaica; it is the size of a pear and splits open, when ripe,

exposing the black seeds and whitish flesh. Both the seeds and the meat of this fruit have been shown to be toxic.[160-162] It is irrelevant to the present discussion whether all cases of Jamaican vomiting sickness represent "ackee poisoning." Let us concern ourselves rather with the hypoglycemic materials which have been found in the akee.

Hassall and co-workers[155-157] extracted from the seeds of the akee two toxic polypeptides which they named hypoglycin A and hypoglycin B. Both compounds were lethal to kittens, guinea pigs, and rats; hypoglycin B was approximately half as toxic as A. Hypoglycemia was a striking pharmacologic property; the blood sugar invariably fell to less than 20 mg. per 100 ml. before death.

Several groups of workers[158, 163, 164] have recently characterized these materials chemically. The general formula may be represented as:



(R) = OH in hypoglycin A
(R) = glutamic acid in hypoglycin B

There is a species difference in susceptibility to the agents. Hypoglycin A or B given intravenously will cause hypoglycemia in the rabbit, monkey, rat, and mouse, but not in the dog, cat, or pigeon.[159,165] Hypoglycin A is more potent than B. Hypoglycin A was orally effective in the rat.

Emesis and hypoglycemia do not appear to be related[159,165] since the pigeon, cat, and dog vomit but the monkey does not. The mouse, rat, and rabbit are emesis resistant, as expected. Depression and prostration were seen in all animals given toxic doses of the agents.

The pancreas does not seem necessary to the hypoglycemic effect since this occurs in the alloxan diabetic rat.[165] An effect has also been shown in the adrenalectomized mouse.[159]

These agents alter liver glycogen content in susceptible species. Hill[152] and Jelliffe and Stuart[153] have drawn attention to the marked depletion of liver glycogen in cases of clinical poisoning. Liver glycogen granules disappear in the treated rabbit and glycogen content decreases in the mouse[159] and rat.[166] Fatty metamorphosis of the liver following lethal doses of hypoglycin A or B has been shown in the mouse, rat, and monkey, and even in the cat, dog, and pigeon, which do not show hypoglycemia.[159] The livers of starved mice were capable of synthesizing glycogen after hypoglycin A or B treatment when glucose was given subcutaneously every 30 minutes.[159]

Pathologic changes other than fatty metamorphosis of the liver seen after lethal doses include necrosis of the thymic and splenic lymphocytes, pulmonary edema, and erosion of the gastric mucosa.[159]

von Holt and Leppla[167] could find no response of the rat diaphragm to hypoglycin A in vitro.

Pretreatment of mice with riboflavin diminishes the effects of and mortality from hypoglycin.[168] It has been shown that hypoglycin increases the rate of oxidation of C^{14} glucose while it inhibits the oxidation of palmitate. It has therefore been suggested that this amino acid acts primarily by blocking fatty acid utilization.[169]

We can conclude at present only that hypoglycin presents interesting possibilities for research into certain aspects of intermediary metabolism. From a clinical standpoint it does not seem to promise much in the field of therapy.

The salicylates

The use of salicylates in the treatment of diabetes goes back to 1876.[18] The first report was confirmed both in Germany[170] and in England.[171] It is evident from these reports that the results were unpredictable and inconstant, but sometimes rather spectacular. Recently interest in this subject was revived in England by Reid and co-workers.[172,173] They observed abolition of glycosuria and attainment of normoglycemia in 7 patients. The doses required, however, produced one or another of the unwelcome

effects of salicylism. Another interesting observation is the seeming potentiating effect of calcium acetylsalicylate when used together with a sulfonylurea.[174]

Blood sugar fall due to salicylates is at variance with observations made during intoxication with these materials. Generally, hyperglycemia is found in man and in animals.[175-179] In the diabetic animal, however, whether as a result of pancreatectomy[180,181] or following alloxan,[182] salicylates reduce the glycosuria and hyperglycemia. In the normal animal the major effect seems to be a reduction in liver glycogen.[182-185]

Salicylates exhibit many effects which could be thought of as playing a role in the above findings, e.g., on adrenal secretions[186-189] and on a variety of tissue enzymes.[183,190] None of these effects gives a rational explanation of a hypoglycemic effect. Perhaps salicylates belong to a large group of pharmacologically unrelated materials which have one effect in common, namely, interference with one or another step at the terminal portion of the respiratory chain. This type of action at the cell level seems to break down the barrier to ready glucose entry into certain tissues.[191] The effect resembles that of insulin, but the mechanism by which it is achieved is hardly physiologic.

The intriguing aspect for the future is research into mechanism and the possibility that small doses of salicylates may permit the use of smaller doses of the sulfonylureas, and thus limit side effects to a bare minimum.

Indications for and clinical use of oral hyploglycemic agents*

The development of such agents as the sulfonylureas and the biguanides has certainly served to enliven a multitude of investigational approaches to diabetes in all of its phases. What of the therapeutic benefits already achieved or likely to appear in the near future? Are there any advantages to oral therapy of diabetes over and above simple convenience? What are the hazards of short-term and of the prolonged use of such hypoglycemic agents? What is the role of diet, insulin, and exercise in the "oral" therapy of diabetes? These and other such problems will be considered in this section with regard to the two presently used groups of drugs, (1) the sulfonylureas and (2) the biguanides, since in the main different considerations apply to the rational use of each of these groups.

Sulfonylureas. These drugs are effective, in the sense of blood and/or urine sugar reduction, in about 65 per cent of diabetic patients whose disease had its onset in adulthood, when they are used in moderate and reasonably safe dosage. In general, the degree of severity of the diabetic state is inversely related to the effectiveness of these drugs. Since the actions of these substances seem to be mediated in the main by the amount of insulin which they help to liberate or potentiate, it is reasonable to expect that they would fail when the patient's pancreas contains a relatively small amount of the hormone compared to the degree of hyperglycemia. Diabetic individuals with "adult onset" who readily develop ketonuria upon insulin withdrawal do not usually respond to the sulfonylureas. Complications such as infections, trauma, operative procedures, etc., which generally tend to intensify the metabolic disturbance and would ordinarily require, temporarily, sizable increases in insulin dosage, cannot be handled with oral medication by itself.

The studies of Wrenshall and Best have shown that the pancreas of "adult onset" diabetic patients contains significant amounts of insulin (approximately 30 to 50 per cent of normal in most cases). Assays for insulin-like activity of plasma demonstrate that subjects with this type of diabetes possess on the average about 70 per cent of the "normal" amounts of "insulin"

*This section represents the considered opinions of the authors and is based upon personal experience and the available literature. There have been many symposia and conferences dealing with this subject. Following are listed some of the publications arising from such meetings which will give the reader a rich field for his own exploration:

I. Sulfonylureas and other sulfonamides.[192-202]
II. Biguanides.[199-202]

in their circulating fluids. In view of the animal experiments cited above it would be reasonable to assume that the "adult onset" diabetic may suffer from an inability to respond to hyperglycemia with an additional output of insulin. It is quite possible that the sulfonylureas enable the beta cell, or the insulin-containing granules within that cell, to respond more briskly and sensitively to a rise in blood sugar. Such considerations, though unproved, are consistent with clinical experience in relation to responsiveness to the drugs. In juvenile diabetes during the first few months following diagnosis the per cent responsiveness is greater than after a period of 6 months has elapsed. Presumably the responsive period corresponds to a time when a sufficient number of beta cells are still in condition to manufacture and release some insulin.

It is of course obvious that the control of body weight and the regularity of food intake are basic to the therapy of diabetes, whether insulin or oral medication be used in addition. Perhaps it might seem superfluous to belabor that point, but one must stress that it is the physician's duty to reemphasize this truism to the patient, since there is a distinct impression among some diabetic individuals that the oral drugs are a ticket to freedom from dietary restrictions.

As is well known, a large proportion of the "adult onset" diabetic patients can be treated successfully by diet alone. There seems therefore in most such instances no purpose being served by administering any of the oral drugs. It may be argued by some that aglycosuria is fairly easily achieved in such cases by dietary management alone, but that normoglycemia is a more difficult aim, and that under these conditions it would be reasonable to enlist the aid of hypoglycemic agents. Whether one does employ the oral drugs depends upon one's conviction of the degree of harmfulness of persistent mildly elevated bloodsugar levels, especially as this relates to the various vascular and neurological complications of the disease.

Responsiveness to the sulfonylureas bears little or no relation to the known duration of diabetes or to the duration of insulin therapy. In general, however, there is a rough negative correlation between the degree of insulin requirement and the response to oral agents of the sulfonylurea type. As an approximation it may be stated that one cannot expect lasting results in patients requiring 40 or more units of insulin per day. This is not true in the individual case, because the amount of insulin a particular patient receives may not be a true index of the severity of the diabetic state or the degree of deficiency of endogenous insulin. Many a diabetic person does not actually *require* the amount of insulin he *receives*.

As was mentioned previously the age at onset of the diabetes is directly related to response. The age of 40 is a useful dividing line between the two major forms of diabetes. At present we have no true explanation why some typical cases of mild or moderate diabetes of "adult onset" "requiring" small amounts of insulin do not respond to the sulfonylureas. We know practically nothing about the biosynthesis, the storage, and/or release of insulin in the beta cell and the regulation of these functions. It is theoretically conceivable that the unresponsiveness of the beta cell is due to inhibition at several points between synthesis and release, and not all of these blocks need necessarily be removed by such agents as the sulfonylureas.

The most reasonable selection of patients for the sulfonylureas are those with onset in adulthood in whom a conscientious trial of good dietary management proves insufficient for good control and who do or would *require* up to 40 units of insulin per day. Coexisting disabilities or complications which would prove to be positive contraindications are few, with the exception of significant dysfunction of liver or kidney. Some disabilities are actually good indications for oral therapy. These are: visual difficulties due either to cataract or to retinal problems; paresis or paralysis of the extremities; tremor of significant extent; mental

defect and severe emotional disturbances.

Three sulfonylurea preparations are now in use, carbutamide, tolbutamide, and chlorpropamide. Of these only the latter two are available in the United States. In most other countries, while carbutamide is also available, there seems to be a shift away from its use during the last year or so. The comparative disadvantage of carbutamide is its tendency toward a greater number of "side effects" especially of the "allergic" or sensitivity type. The most alarming were jaundice due to intrahepatic cholangiolar obstruction; leukopenia and agranulocytosis and a generalized "vascular" reaction coupled with myocardial necrosis. Another of the sulfonylureas which was subjected to trial was metahexamide. Experience similar to that with carbutamide led to suspension of further trials. It is significant that both of these substances possess an NH_2 group on the benzene ring of the molecule, while tolbutamide and chlorpropamide do not. This structural difference also accounts for the fact that carbutamide shares the antithyroid activity of the class of sulfonamides while tolbutamide and chlorpropamide have a much less significant action in this respect.

None of the sulfonylureas has led to crystalluria and consequent kidney damage. Their excretory products are all very soluble in water and biologic fluids. In man, carbutamide appears in the urine mainly as the acetyl derivative; the principal metabolite of tolbutamide is the inactive carboxyl derivative; and chlorpropamide is excreted unchanged.

In the dog the metabolism of the sulfonylureas may give rise to hepatotoxic intermediates. This is presumably the reason for liver damage, including lowering of the prothrombin level, which is seen following chronic tolbutamide administration to the depancreatized dog. This does not seem to be the case in the human as attested by numerous series of cases in which liver function tests were performed periodically over prolonged periods.

In a significant number of diabetic individuals (5 to 10 per cent) who prove to be responsive to the sulfonylureas, the effectiveness may diminish or disappear 3 to 6 months following initiation of therapy. This phenomenon of "secondary failure" has not received an adequate explanation as yet. Substitution of one of the drugs for another member of the group has been reported to evoke anew the original type of response. A period of rest from the drug (with the interim use of insulin) has also been of value in restoring effectiveness.

While this is not the place to outline procedures for the clinical use of the sulfonylureas, it should be pointed out that the maintenance dose of tolbutamide ranges from 1.0 to 2.0 Gm. per day and that of chlorpropamide from 250 to 500 mg. While larger doses than those mentioned can be tolerated the adherence to the given range seems to be best calculated for minimal incidence of untoward effects.

The attempts to use oral drugs in conjunction with insulin in cases in which oral therapy by itself is not effective seems in most instances inappropriate and of little value. It has been claimed that such combined therapy leads to "smoother" control of "difficult" cases. The variability encountered in the same patient over periods of time in diabetes is such that the evidence for "smoother" control is difficult to demonstrate.

The biguanides. If one sums up the experimental and clinical observations concerning the actions of the biguanides, one is faced with a seeming paradox which makes many clinicians hesitant in the use of these drugs in diabetes. The experimental data point to the fact that these compounds inhibit the activities of one or more of the enzyme systems at the terminal end of the respiratory chain—at about the level of the cytochromes. It is therefore thought that this inhibition of the oxidative chain engenders an increase in anaerobic glycolysis as well as a loosening of the barrier to glucose entry located at the cell membrane. In the liver, gluconeogenesis, which requires a high oxidative

activity, is reduced in rate—hence hypoglycemia. This lowering of the blood sugar is independent of the integrity of the beta cells and of the presence of circulating insulin. Hence the biguanides are active in the diabetic animal and in the juvenile human diabetic patient.

If this be the explanation of the mode of action of these drugs one would expect at first glance a general deleterious effect on many bodily functions following their chronic use. One would hardly expect unalloyed benefit from chronic depressions of the oxidative capacity of the cells. It is therefore somewhat of a paradox that the patients (who are able to tolerate the ingestion of the drug) appear not to suffer from such expected deterioration. Tests of liver, kidney, bone marrow, and other functions remain unaffected by biguanide medication. The explanation may be in either of two directions. The biguanides, in therapeutic doses in vivo, may lower blood sugar by mechanisms other than those adduced from animal experimentation or the inhibition of oxidative metabolism may be small, periodic, and completely and quickly reversible, so that no cumulative deteriorating effects are obtained.

Two types of undesirable side effects emerge in the use of the biguanides. About half of the patients tested have to discontinue the drug because of irritative gastrointestinal phenomena. The group which is able to tolerate the continuous use of these drugs seems to fare well with the exception of some individuals who develop a feeling of weakness and a loss of appetite.

Perhaps the closest one can come to an indication for the use of biguanides at present is the difficult case of brittle diabetes which swings from the depths of hypoglycemia to the dangerous heights of extreme hyperglycemia and ketosis. The biguanides in small doses seem able to smooth the course and make good insulin control feasible.

More definitive studies on the mode of action of the biguanides are urgently needed if we are to decide in a rational manner on their place in clinical pharmacology.

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We shall continue to experiment and try new drugs; therapeutic miracles will follow one another at an increasing rate. Will we pay too high a price for this progress? Will we sacrifice human beings unnecessarily? The answer depends on the medical profession as a whole and, even more important, on the individual physician. "Man is a prober and a meddler, and in this, so long as he holds true to his own gifts, he will not stop" (H. J. Muller, Scient. Month. 84:254, 1957). Among the physician's gifts must be the ability to grasp broad concepts, to reflect and ponder, to advance from the first learnings to new, more complete knowledge and understanding. In a word, he must employ therapeutic agents with wisdom, in such a way as to assure the realization of maximal benefits, exercising all the reasonable precautions against the occurrence of undesirable effects and iatrogenic syndromes. He must produce miracles without mischief—a superhuman task facing the one man in our culture who is conceded superhuman powers.

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Psychopharmacologic theories: a critical review

Understanding of the actions of the new psychopharmacologic drugs must depend upon the development of a theory which relates biochemical or pharmacologic events to behavior. On the biochemical side the problem is formidable and is aggravated by the lack of adequate behavioral referents. Theories constructed by biochemists and pharmacologists may be psychologically naïve, while those proposed by behavioral scientists may greatly oversimplify their treatment of physiologic complexities. A particular difficulty is presented by the ambiguous and subjective psychological terms used in many theories. No single theory so far is consistent with all the empiric data.

The majority of studies of the new psychotropic drugs utilize clinical ratings or descriptions on the behavioral side. These studies and those of experimental psychosis suggest some generalizations about drug effects. When specific and objective measures of behavior are used—measures like reaction times, psychophysiologic responses, and rate of conditioning—many contradictory findings emerge. The clarification of these contradictions will require a complex yet integrated theory of behavior. The new psychotropic drugs can serve as an invaluable class of independent variables to aid in the development of such a theory.

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Current-day psychopharmacology needs some good theories. We have drugs that can do a variety of things to human behavior, and we have some theories about the effects of these drugs at the physiologic level.[49,66,77,79,88,102,108,127] Unfortunately, psychopharmacology is concerned with what drugs do to complex behavior—to mood, emotion, communication, learning, and the like. What a drug does to serotonin is interesting, and it is nice that some models of the neuron now allow us to translate statements about serotonin into statements about electrical potentials in the brain. But we have few models of the mind that allow us to translate statements about serotonin into statements about complex psychological behavior.

This article reviews various observations on how drugs influence behavior—and how various models and theories attempt to make these observations assume some order and closure. A few heroic theories attempt to capture both physicochemical and psychological observations with a single model. Others struggle with the more modest but

brave task of finding some lawfulness among just the psychological observations.

None of these model-builders are completely successful, yet all have at least stimulated other workers. In spite of shortcomings, these theories and models are worth studying in their own rights. Some may contain the germs of more general propositions, and all may help us convert pitfalls of the past into guideposts of the future.

I. Theories suggested by the hallucinogens

A. A chemical theory with psychosis as the critical behavior. Perhaps the most seductive and dramatic behavioral effects are those produced by the hallucinogens. Since psychosis is often accompanied by hallucinations, the hallucinogens have been widely hailed as a new key to understanding both psychosis and the action of drugs used in the treatment of psychosis. Several notable theoretical structures have already been constructed on this basis.

The fundamental behavioral data seem quite unambiguous at first glance. Certain substances do indeed produce dramatic behavioral alterations which mimic clinical psychosis. Granting certain differences between laboratory psychosis and clinical psychosis, still the hallucinogens may contain a clue to chemical factors that play a clinical role. But is psychosis the proper behavioral referent to consider?

Take for example the theoretical proposal put forward by Hoffer and Osmond.[67] These workers have documented their theory at some length and have selected 106 references to support their position. Here, a summary of this elaborate theory must serve the purpose of our discussion. Many of the hallucinogens are indoles or may be indolized in vivo. The list of indoles or indolized substances which can produce hallucinations is long and includes LSD-25, mescaline, bufotenin, dimethyl- and diethyltryptamine, trimethoxyphenylaminopropane, and certain Mexican mushroom hallucinogens. Since indoles may be formed in the degradation of naturally occurring neurohumors, the body fluids of schizophrenics were studied for evidence of indolic substances derived from epinephrine. Since adrenochrome and adrenolutin are considered to be oxidative products of epinephrine metabolism, they were studied as possible hallucinogens.

Hoffer and Osmond collected evidence from which they inferred that some oxidative product of epinephrine (presumably adrenochrome or adrenolutin) produces schizophrenia-like hallucinations in human subjects. Other workers[61,62] have suggested that schizoprenic individuals have an abnormal amine metabolism, due to "taraxine." Such a defective oxidative mechanism might be involved in the production of toxic indole metabolites of epinephrine. Finally, many workers[91] report that the body fluids of schizophrenic subjects seem to be toxic in a variety of situations.

All in all, Hoffer and Osmond do a good job of implicating a defective oxidation of amines in schizophrenia. This postulated amine oxidation defect *might* produce abnormal metabolites of adrenaline which could act as schizophrenogenic substances. Such substances accumulating in the body fluids of schizophrenic persons *might* help produce abnormal metabolites of adrenaline which could act as schizophrenogenic substances. Such substances accumulating in the body fluids of schizophrenics *might* help produce symptoms of schizophrenia (either directly or as some link in a longer, more complex chain of events). Such substances *might* also be responsible for the toxic effect of body fluids from schizophrenic individuals observed by other workers.

Hoffer and Osmond report that phosphate excretion in normal persons is altered by both adrenolutin and LSD-25 to resemble the phosphate excretion in schizophrenia; and that substances which antagonize psychotomimetics tend to be therapeutic for schizophrenia, whereas substances which potentiate psychotomimetics are not. These authors thereupon conclude that adrenochrome and adrenolutin are factors in

certain forms of schizophrenia. Finally, they write, "Treatments based on the adrenochrome hypothesis have shown therapeutic success for early schizophrenic patients."[67] This "adrenochrome hypothesis" has played a role in stimulating many studies, but there are now numerous reasons for questioning its adequacy. The biochemical basis for this theory has been amply criticized by Kety.[77] Considerations of diet, activity, occult infectious disease, drugs, and the like, confuse most studies on the metabolism in schizophrenia. LaBrosse, Axelrod, and Kety[81] report that o-methylation rather than oxidation is the principal route of metabolism for epinephrine in man, although the data of Resnick and associates[107] suggest that oxidative breakdown of adrenaline does occur. Kety also notes that "Sizer, Axelrod and Perlin using techniques of high sensitivity were unable to detect adrenochrome in the blood of normal test subjects or in that of acute or chronic schizophrenic patients."

There is also reason to raise some questions about the psychological effects of adrenochrome on human subjects. Whether this substance is a hallucinogen, a psychotomimetic, or just "a psychologically active" drug is far from clear. Both the problem and some conflicting viewpoints are clearly brought out in the exchange between Osmond and Hoffer[100] and John Benjamin.[6]

The utility of the drug-induced psychosis in testing therapeutic drugs has been questioned by Isbell[73,74] He reports that chlorpromazine effectively blocks the hallucinogenic effects of LSD-25, whereas reserpine does not; he therefore concludes that LSD-25-induced psychosis is not useful in the screening of antipsychotic drugs since it fails to demonstrate the antipsychotic effect of reserpine.

On the basis of these data, one can question: (1) whether the oxidative metabolism of epinephrine postulated by Hoffer and Osmond is actually significant in human subjects; (2) whether adrenochrome and adrenolutin do, in fact, induce psychosis-like symptoms; and (3) whether drug-induced psychoses are useful models for antipsychosis drugs.

Finally, we may ask whether or not there is any special relationship between the possession of an indole nucleus and the ability of a compound to produce hallucinations. It is of interest that the Russians use an atropine-induced psychosis as their model for schizophrenia, an approach thoroughly documented in a compilation of papers edited by Goldenberg.[55] Ostfeld and co-workers[101] used JB 318 (n-ethyl 3 piperidyl benzilate) to produce hallucinations in human subjects. There is also the work of Lubin and co-workers[86] on Sernyl (phenyl cyclohexyl piperidine) described as "unrelated to other psychotomimetic agents" and originally introduced as a sensory blocking agent for anesthesia. Neither JB 318 nor Sernyl contains the indole rings.*

All these arguments—pro and con the adrenochrome hypothesis—are interesting, but what can they possibly contribute to psychopharmacology? Perhaps when the smoke clears we will know more about amine metabolism, but will we be in any position to relate this to behavior? At best it seems that adrenochrome may have something to do with some clinical psychosis. The term psychosis connotes little more than severe mental disturbance, and so we may eventually be able to say that altered amine metabolism is a factor in the illness of some people who are more than a little psychologically disturbed. Obviously, amine metabolism will not be the only factor in such a broadly defined syndrome, and so at best it will take its place with a host of other toxins (symbolic as well as physical) that make people sick enough to be called psychotic.

This sort of approach overlooks the most important aspect of the hallucinogens. They do not just make people sick. They make people sick in a most unusual way. Unfortunately, it is hard to describe unambigu-

*The reader whose curiosity is stimulated by this brief comment on the general topic of hallucinogens will find an annotated bibliography on LSD-25 published by the Sandoz Pharmaceutical Company.

ously just what constitutes the unusual aspect of the behavior disorders they produce. It is easy to take scientists to task for thinking that they have been explicit when they have used vague words like "psychologically active," or "psychotomimetic." In fact, it is much easier to point out such fallacies than to offer anything better. Yet in spite of the inherent difficulties, there are more specific and measurable aspects of psychological function that can be isolated for special attention. These more discrete functions allow more repeatable psychological observations on drug effects and they carry with them the possibility of a bonus in the form of increased fundamental knowledge.

B. Studies of more subtle changes induced by the hallucinogens (the body-image approach and others). To illustrate the use of drugs in studying the more subtle psychological changes, the concept of the body-image is apropros. The concept combines a definite meaning with inherent vagueness typical of so many behavioral constructs.

The body-image may be considered the mind's model of the self in relation to the outside world. Information concerning parts of the body, the position of the body in space, and the ability of the body to operate on the outside world are all essential to adequate functioning in the mature human being. This body-image furnishes a basis for predictions about the outcome of proposed actions and allows the organism so to use the inherent redundancy of sensory data as to reduce the load of information that it must process. This complex model or body-image is often distorted in clinical conditions and appears altered in certain drug states.

The body-image is apparently quite flexible and must be maintained by a continuous input of relevant sensory data. Perceptual isolation reduces meaningful sensory input and results in disordered psychological functioning. This procedure[116] can result in hallucinations and psychosis-like episodes. There is also evidence that disturbance in the body-image results from the administration of hallucinogenic drugs. Physiologic evidence[9,10,49] suggests that LSD-25 can interfere with sensory input, and perhaps part of the effect of LSD-25 is due to this interference with the sensory data required for maintenance of the body-image. Sernyl, the hallucinogenic drug mentioned above, seemingly produces a sort of pharmacologic sensory deprivation and may exert its psychological effect by means of "a desynchrony or defect in proprioceptive feedback."[86]

Psychological data were collected by Silverstein and Klee,[114] who studied drawings made by normal subjects without the drug and at the height of the reaction to LSD-25. The drawings made under the influence of LSD-25 differed distinctly from those made without it; and the nature of these differences can be taken to indicate that LSD-25 does in fact influence the body-image.

Another approach has been adopted by Fisher.[45] Presumably the body-image becomes progressively differentiated during the maturation of the individual. Fisher discovered in right-handed individuals, as they mature, a relative increase in the galvanic skin response on the nondominant side. Thus a right-handed adult will have greater galvanic skin responses in the left hand than in the right hand. Fisher finds that this differentiated galvanic skin response pattern is missing in schizophrenic patients and that it also breaks down in normal subjects after the administration of LSD-25.

An even more complex view of the relationship between the self and the surrounding world is found in the sensory-tonic field theory. Liebert, Wapner, and Werner[85] write, "According to sensory-tonic theory, perception is held to be not simply a function of stimulus conditions, but rather the function of the relations obtaining between the impinging stimulus and the ongoing organismic state. From this it follows that a change in either external stimuli or the organismic state will produce a change in perception" (p. 193). As the individual develops he progressively differentiates himself

from his environment and is able to view things as distinct entities in themselves. In immature individuals, whether children or adults who are regressed or caused to regress by drugs, one should be able to see effects on this differentiation of the self.

To test the differentiation of the self, these workers place the subject in a darkened room in a chair so arranged that it can be tilted. At the beginning of an experiment the subject is seated in the chair, which is tilted to one side, and he is presented with a luminous rod that is slightly off vertical. He is then instructed to adjust the rod until it is truly vertical. Under such a condition, children say that the rod is vertical when it is still tilted to the side of their body tilt. But with increasing age the subject's judgment of apparent vertical moves across the true vertical until, in the adult, the apparent vertical is tilted slightly away from the side to which the body is tilted.

The initial position of the rod also influences behavior. In general, children tend to perceive the rod as being vertical when it is still tilted in the direction of its initial position. As children mature, this tendency becomes less and less marked.

With respect to this tendency, schizophrenic adults behave more like children. Their perceived vertical is tilted slightly to the side to which their bodies are tilted and more in the direction of the initial position of the rod. LSD-25 was expected to have a similar "primitivizing" effect on normal subjects and, for the starting position, the experiment bore out this prediction. Thus, for normal as well as schizophrenic subjects, LSD-25 increased the effect of the starting position and caused subjects to judge the rod to be vertical when it was tilted away from the plumb line in the direction of the starting position. Although this effect was not significant in normal subjects, the change induced by LSD-25 in schizophrenic persons was highly significant. The body-tilt effect results did not support the hypothesis. As mentioned above, schizophrenic patients, like children, tend to adjust the apparent vertical so that it is actually tilted to the side to which the body is tilted, while normal persons adjust the rod to the side opposite to which the body is tilted. LSD had no influence on the body-tilt effect in schizophrenics and caused normal subjects to displace the rod significantly more in the direction opposite to the body tilt. Thus the sensory-tonic field theory fails to make adequate predictions in certain situations but does suggest experiments within a logically coherent framework and allows the collection of repeatable data.

The concept of the self in relation to the environment is not the only psychological variable that differentiates schizophrenic from normal subjects. For example, schizophrenic patients show impaired memory for visual images only when the image to be memorized is exposed for a relatively long time. Under conditions of short exposure, their memory for visual images does not differ from that of control subjects. Brengleman, Laverti, and Lewis[19] found that LSD-25 impairs memory for visual images only when the image to be memorized is given a long exposure. Thus such subtle changes can be isolated, measured, and studied even in a field like perception where gross psychosis-like changes often blind the observer to subtle effects.

II. Drugs and emotions

A. The influence of drugs on clinical and introspective evaluations of emotion and mood. The recovery of a chronically psychotic patient coincidentally with the administration of some drug is as dramatic as the opposite phenomenon—the drug-induced psychosis. Recovery, however, is an even less satisfactory foundation for a psychopharmacologic theory than is psychotogenesis.

Foulds,[48] for example, surveyed in the British and American literature the clinical studies of psychopharmacologic agents, tabulating: (a) whether controls were used in the experiment, and (b) whether the therapeutic trials were successful or unsuccessful. By far the greater number of suc-

cessful trials were reported when no controls were used. Tabulating use of controls versus success, Foulds found a chi square of 21.06 with a p value of less than .001. On this basis one would expect that a large number of successful clinical trials reported in the literature simply represents the lack of adequate controls.

Even with controls, the presence or absence of psychosis and other gross indices of clinical condition are too general and too vague to support an adequate theory. This is the same criticism that was leveled against the elegant adrenochrome hypothesis and again it seems that more critical and definitive behavioral variables must be isolated for study.

Especially in the area of psychopharmacology, a major avenue of approach to understanding the effects of drugs is the examination of their effects on emotional behavior and on motivational factors generally.

As might be expected, great variation is found in the degree of precision and specificity with which the behavioral effects are described. Observations range from reports of clinical improvement with tranquilizer therapy in conditions described as "anxiety" or "tension," measures of the physiologic component of emotional responses following drug administration.

For example, many clinical studies report the relief of anxiety and tension by the phenothiazines or by Rauwolfia derivatives.[11,95,113,121,128] Such studies indirectly support the belief that the tranquilizing drugs can affect emotional responses. Segal and Shapiro,[112] however, have reported that a 3 week course of reserpine produced no greater improvement than did a placebo in a double-blind study on "outpatients who showed anxiety and/or its manifestations."

Chlorpromazine also appears to affect the response to pain. Corbit[34] found that in postoperative gynecologic patients the intravenous administration of 25 to 50 mg. chlorpromazine led to the decreased use of narcotics. Cole and Robertson[33] gave 50 mg. chlorpromazine in combination with phenobarbital or chloral hydrate to patients with tetanus, and stated that chlorpromazine spared the patient "much of the anxiety and physical pain due to the spasms." In a study by Houde and Wallenstein,[69] however, chlorpromazine (25 mg.) was reported to have no analgesic power either alone or in combination with morphine when given to hospitalized cancer patients with severe pain.

Beecher[3,4] has emphasized the concept of "reaction component" in subjective responses to pain, arguing that analgesic drugs such as morphine do not affect the sensation of pain or the pain threshold. Rather, he feels, they affect the patient's reaction to pain based on an interpretation of its significance. Chlorpromazine's ineffectiveness in the cancer patient raises some question as to whether the mechanism of this drug is similar to that of the true analgesics. In both instances where chlorpromazine was found to reduce pain, there was some physical basis for its effect. In Corbit's study, this drug was given originally for its antemetic effect and its reduction of postoperative nausea may have been responsible for the reduction in discomfort experienced by these patients. Cole and Robertson[33] stress the value of chlorpromazine in relaxing the actual muscle spasms in their tetanus patients, and so the reduction of the physical symptoms may have been responsible for the reported decrease in anxiety and pain. Indeed, the beneficial effect that the tranquilizers have on some anxious patients may be secondary and due primarily to their ability to reduce the physiologic symptoms which arise from and then further aggravate these patients' anxiety. This inference is suggested by the observation of Sarwer-Foner and Ogel[110] that when the symptoms serve as part of the patient's defense mechanism, a tranquilizing drug that eliminates the patient's symptoms may actually aggravate the anxiety and tension in the case.

Benactyzine, which, according to Jacobsen and his co-workers,[75] has a specific effect against anxiety and fear in animals, has been reported by such investigators as Ray-

mond and Lucas,[104] Davies,[35] and Hargreaves and associates[59] to be valuable in the treatment of human anxiety and tension states. Kinross-Wright and Moyer[78] reported that the main effect of this drug was "reduction [illegible] notional reactivity to stress." Hargreaves, [illegible]ilton, and Roberts,[59] using a double-blind technique, obtained a semi-objective anxiety score based on ratings by psychiatrists of 13 psychological and somatic indicators. During the 3 week administration, benactyzine produced nearly twice as great a reduction in the anxiety score as did a placebo.

While reports of specific reduction of anxiety by drugs are scarce, those concerning production of euphoric states by drug administration are more easily found. Although the reduction of anxiety may seem to be associated psychologically with the production of euphoria, the reverse relationship may hold at a pharmacologic level. That is, many drugs that produce euphoria in some subjects in some circumstances produce anxiety in others or in the same subjects under different conditions. Besides the amphetamines, which produce euphoria and anxiety or both, several other substances have been reported to have similar effects: trimethoxyamphetamine,[103] 2-dimethylaminoethanol,[96] pipradrol,[57] and LSD-25.[54] This group of compounds all belong to the class of drugs that are stimulant or hallucinogenic or both. Euphoric effects are also obtained, of course, from some depressant compounds, notably ethyl alcohol, morphine, and other opiates and the barbituates. Marley and Chambers[89] have reported a similar type of euphoria from methylpentynol, while Boswell,[12] Fleminger,[47] Hench and collaborators,[63] and Irons and his associates[72] reported euphorigenic effects from cortisone and ACTH on some patients.

The significance of euphoria as an emotional response is not at all clear. The recent work by Olds and Milner[98] and by Miller[92] and Brady[15] on "reward" or "pleasure" centers in the brain may soon lead to improved understanding.

From the material presented in this section, it is apparent that drugs do influence emotion. But clinical descriptions of such changes leave much to be desired.

In spite of the inherent difficulties in dealing with introspective evaluation of emotion, Wendt and his associates at the University of Rochester have made considerable progress. Their work on a measuring of subjective or introspective responses in an objective way has been summarized in a paper by Nowlis and Nowlis.[97] Of their numerous techniques in studying the effects of drugs upon their subjects, the method most relevant to the question of emotional effects of the compounds was their adjective check list. This self-rating device consisted of 100 to 200 adjectives to which a subject responded rapidly. Four response options were permitted to indicate the degree to which each adjective applied to the subject at the moment when the self-rating was being performed. Significant and consistent shifts in self-description were obtained after administration of drugs. For instance, Dramamine produced a definite increase in the checking of such words as "tired," drowsy," and "detached"; and a definite decrease in the checking of those such as "business-like," "genial," "industrious." Benzedrine, on the other hand, typically increased the checking of words such as "business-like," "talkative," "capable," and decreased the use of those such as "lazy," "languid," and "nonchalant."

Testing was ordinarily conducted with the subjects in groups of 4, one group on each experimental day. The composition of the group, in terms of the drugs they had taken, markedly affected the response to the drugs. For example, when all members of a group had received secobarbital, the adjectives showing greatest increase in frequency of selection were such words as "expansive," "forceful," "courageous," "daring." But if each of the 4 had taken a different drug—secobarbital, Benzedrine, Dramamine, and lactose—the words showing the greatest increase in selection by the subject who had taken secobarbital were

such as "distractable," "dizzy," "drifting," "glum," "defiant," etc. These differences, first observed haphazardly, were later carefully documented in a factorially designed experiment.

The approach just described seems to promise a truly objective approach to problems of subjective reactions. The data, however, are of somewhat unwieldy form, since they consist of percentage increases or decreases in choice of as many as 200 separate adjectives. The results of the factor analysis of responses to these check lists, in progress at the time of the Nowlis and Nowlis report, have not as yet been published.

The semantic differential technique of Osgood, Suci, and Tannenbaum[99] should also be applicable to an attempt at measurement of emotional changes as a result of drug administration. Such an application has not so far been reported.

In summary, the clinical studies which specifically question whether tranquilizers reduce clinical anxiety have for the most part yielded affirmative answers. Taken in conjunction with the dramatic benefits which these drugs have brought to the treatment of psychotic patients, such findings have led to a widespread acceptance of the notion that the tranquilizing drugs produce their effect by reducing anxiety. However, this belief is not confirmed in a clear-cut way by experimental studies which attempt to evaluate objectively the effects of these drugs on emotional behavior. Limited attempts to utilize the verbal reports of subjects about their feelings after administration of a psychotropic drug show generally depressant or stimulant syndromes; but the most outstanding demonstration in these studies has been that the subject's prevailing social situation can produce virtual reversals in the drug effects.

B. Drugs and bodily changes that accompany emotion. If emotions are so difficult to describe, perhaps the bodily changes that often accompany emotion would be better behavioral indices to use in studying drugs.

A wide variety of bodily changes occur during or following emotional responses. These changes have been extensively studied and reviewed by Altschule,[1] Dunbar,[39] and Gellhorn.[53] Although such indices of emotional response can be obtained rather easily, they have been used surprisingly little in evaluating the effects of drugs. One widely used measure of emotional response already mentioned, the galvanic skin response (GSR), was employed by Elithorn and associates[40] to evaluate the responses of patients who have had leukotomies to painful stimuli; it showed that the anticipatory fear response was reduced, while the response to actual pain was unchanged, in comparison with the responses of control subjects. Carran[30] studied basal skin resistance in two groups of psychotic patients, one group receiving a tranquilizer, the other group no pharmacotherapy. The subjects, tested before and during stress by a flashing visual field, differed in two respects: The drug group had (1) a higher basal skin resistance during the relaxation periods (a difference compatible with the theory that the tranquilizers reduce emotionality); and (2) a greater change in the direction of increased skin resistance during the 10 minute relaxation period before stress began. The two groups did not differ in the response to the visual stress.

Callaway and Dembo[28] found in their study of the electromyographic response to loud sounds that the increased myographic potentials occurring in the half second after each loud sound were reduced by amyl nitrite, nerve gas, and methamphetamine. The reduced response would be interpreted conventionally as an indication of reduced emotionality. Since the stimulants are usually considered to increase emotionality, the decrease in response after administration of the stimulant methamphetamine was surprising. The investigators account for it in terms of attentional, not emotional, factors.

Callaway and Dembo also studied the galvanic skin responses to a horn blast, a flash-bulb flash, and word associations be-

fore and after injection of s[illegible] amphetamine. The basal [illegible] creased following injection [illegible] or methamphetamine—some[illegible] the methamphetamine. [illegible]nductance changes in response to these stimuli were larger after injection of saline than they were under control conditions, and smaller following injection of methamphetamine.

A widely used index of autonomic response is heart rate. While no studies of change in heart rate in humans in response to emotion-provoking stimuli after drug administration have been reported, we may make inferences from two recent experiments: (1) Bersh and co-workers[7] reported a conditioned decrease in heart rate during an emotion-producing stimulus in man; and (2) Wenzel[123] showed that a similar conditioned decrease in heart rate in cats was unaffected by administration of reserpine.

These studies indicate that the autonomic component of emotional response, at least, is not always affected by drugs as would be predicted from their effects on the resting autonomic system.

Jacobsen and his associates,[75] however, found that a 7 day administration of benactyzine led to a general decrease in autonomic response of psychoneurotic subjects during stressful interviews. Bray and Funkenstein,[17] measuring "reactivity of the central sympathetic nervous system" in 15 healthy male college students, found that chlorpromazine increased and prolonged the hypotensive response to mecholyl, a change compatible with the hypothesis that chlorpromazine, by reducing central sympathetic activity, allows a relative predominance of parasympathetic centers. But these authors point out, "on the basis of current information, it is equally possible that chlorpromazine might increase the reactivity of the parasympathetic-epinephrine releasing centers." Greiner and Burch[58] report that in humans undergoing performance and behavior tests, GSR was affected by ether, strychnine, pentobarbital, and pentylenetetrazol (Metrazol) in a manner that paralleled depression or augmentation [illegible]tivity recorded from the reticular acti[illegible]ng system of animals after administra[illegible]on of the same drugs. While these drugs do not overlap with those used by Carran and by Callaway and Dembo, the findings seem rather contradictory; for emotional response is commonly accompanied by arousal of the electroencephalogram.

True, the fact that some drugs produce arousal, with which emotional responses are associated, does not necessarily mean that the arousal-producing drugs should increase emotional responses. Silvestrini and Longo[115] describe an experiment indicating that the arousal response is not a unitary phenomenon, since morphine selectively depressed the arousal response due to painful stimuli but did not alter the arousal to nonpainful sensory stimuli. Scopolamine and pentobarbital, on the other hand, blocked the sensorial arousal more than the nociceptive arousal. Perhaps there are at least three distinct arousal systems: one based on sensory input, one on painful input, and one on conditioned emotional input. It is of interest that Brücke and Stumpf[25] have proposed, on pharmacologic grounds, three different types of arousal reactions in rabbits.

Chlorpromazine and, to a lesser degree, promazine do increase the proportion of alpha activity found in the resting electroencephalogram of man, according to Bruck[24] and Jorgensen and Wulff.[76] Furthermore, Wilson and Glotfelty[126] reported that promazine reduced clinical anxiety in their subjects in response to experimental stimuli and also reduced the EEG arousal response to a flashing light. These results accord with findings in animal studies, which are well reviewed by Bovet and his collaborators.[13] Evidently at least some of the tranquilizers do affect the emotional arousal response of the EEG in the manner to be expected.

Some time ago, Cleghorn and others[32] found that anxious patients showed decreased eosinophils and lymphocytes, and increased neutrophils and uric acid/creatinine ratios in comparison with nonanxious patients. Several groups have demon-

strated a sharp decrease in circulating eosinophils in normal subjects under emotional stress, for example, Humphreys and Raab,[71] Renold and co-workers,[106] Dreyfuss and Feldman,[38] and Markkanen and his associates.[87] The eosinopenia of emotionally or physically stressed rats was prevented by administration of barbiturates, according to Sayers and Sayers[111] and Recant and collaborators.[105]

Kothari and Rindani[80] found that the eosinopenia, which normally occurred in "nondisturbed mental patients" stressed by a single electroshock treatment, was in large measure blocked by prior administration of a reserpine-free extract of *Rauwolfia serpentina*. These authors point out, however, that reserpine itself had a mild stimulating effect on the adrenal, according to the work of Gaunt and his colleagues.[52]

The eosinopenic response is presumed to be mediated by release of adrenal cortical hormones. The demonstration by Mason and co-workers that chlorpromazine and reserpine elevate plasma levels of 17-hydroxycorticosteroids under resting conditions[60] and in response to emotional stress[90] therefore strongly suggests that these drugs would not depress emotionally induced eosinopenia. A direct study, however, of the effects of tranquilizers on the eosinopenic response would be of considerable interest.

Thus, studies of the effects of psychotropic drugs on physiologic indices of emotional response have rather consistently shown that the broad class of tranquilizing and anxiety-reducing compounds produces a preponderantly parasympathetic shift in the resting state of the autonomic nervous system, together with a shift toward less EEG and behavioral arousal. Drugs of the stimulant and antidepressant classes have, in general, the opposite effects, that is, a shift toward sympathetic predominance of the autonomic system and toward greater arousal. Studies of autonomic or electroencephalographic responses to sudden, emotion-provoking stimuli, however, seem to indicate a more complex situation than clinical studies suggest.

We have cited several studies the findings of which do not agree with current theory. For example, the GSR and the conditioned bradycardia failed to respond to administration of tranquilizers. Likewise a stimulant reduced the GSR response to some emotion-provoking stimuli. However, investigations of the effects of sudden stimulation on the EEG arousal uniformly showed a depressant effect of the tranquilizers upon this response. Therefore, peripheral autonomic responses and EEG arousal responses do not seem to be interchangeable measures of emotionality.

Clearly, no royal road to the measurement of drug-induced changes in emotions is afforded by bodily change. Certainly the drug may influence the emotion, and the emotion may influence the body. But the drug may also directly influence the bodily change being studied, and it may also modify the effect of emotion on the bodily change without modifying the emotion itself. These and other sources of confusion limit the usefulness of bodily changes as indices of drug effects on central states themselves. On the other hand, such complexities must be faced and dealt with by any comprehensive psychopharmacologic theory.

C. Drugs and performance disrupted by emotional stress. Another way of evaluating the effects of drugs upon anxiety is to disrupt test performance by introducing stressful or anxiety-provoking factors into the test situation. An anti-anxiety effect or pro-anxiety effect of a drug can then be assessed by measuring the amount of disruption with and without drug administration. Brady,[16] Weiskrantz and Wilson,[122] and Hill and his associates[64] have made good use of this approach with animal subjects. Using human subjects, Hill and co-workers[65] showed that morphine decreases the overestimation of pain intensity ordinarily found in anxious subjects but does not impair the pain intensity discrimination of nonanxious subjects.

DiMascio and Brown with their colleagues[23,36,37] tested several tranquilizing

compounds, utilizing the paired associate learning technique of Spence and co-workers[117] in which the reduction of anxiety was shown to improve learning. In their first study of reserpine, these investigators[23] found that this drug did not relieve subjective anxiety but did impair learning. In later experiments[36,37] using more subjects, they found again that reserpine impaired learning but this time produced a decrease in subjective anxiety. Meprobamate also reduced the subjective anxiety but improved the learning performance, while phenyltoloxamine improved the performance without affecting subjective anxiety. Secobarbital had no effect on anxiety, but impaired performance.

Human subjects were required to perform a perceptual motor task under anxiety-producing conditions by Holiday and Dille.[68] They found that in groups treated with chlorpromazine, pentobarbital, and placebo, learning of the task was significantly disrupted when a loud sound, shock, and air blast were presented as a punishment for certain randomly selected errors. A group tested with meprobamate "exhibited a continued improvement in performance or continuing capacity for learning over successive trials."

In general, if subjects in experimental conditions are continuously subjected to stress during performance of their task both physiologic and behavioral measures of performance may be affected by tranquilizing compounds. But there is evidence to indicate that the most potent of these, such as the phenothiazines and reserpine, impair psychological performance despite their reduction of reported subjective anxiety. The most consistently beneficial substance in these stressful situations seems to be meprobamate, a compound the effects of which on either the autonomic resting state or on autonomic responses to acute emotion-provoking stimulations are virtually undemonstrable.

In the evaluation of studies on so-called objective measures of performance, one caution should be continually borne in mind. The objective performance measures are not subject to social and milieu effects that are the subjective or introspective reports of a subject. This point is nicely demonstrated in a study reported by Starkweather.[118] He divided his subjects into pairs. The subjects were given a drug and tested individually. Following this test, the pairs did a task together. Finally, the subjects were again tested by themselves. The individual testing was thus timed in the hope that the interaction with the partner would affect the results of the second test.

The testing prior to the partners' interaction showed the predicted differences between the two drugs, amphetamine and phenobarbital: faster performance followed the stimulant drug and slower followed the depressant drug. After the cooperative effort, however, a strange thing was noted. The stimulated subjects (who had showed fast performance at the first testing) were slowed to the level of depressed subjects after working with another stimulated subject. But if they worked with a depressed partner, they were at least as fast or even faster on the second testing. The depressed subjects were not further depressed by interacting with a depressed partner but speeded up to a stimulation level. Yet if depressed partners worked with a stimulated partner, they stayed just as slow as before. In these experiments the social effects overcame and even reversed the predicted drug effects.

D. Effects of drugs on conditioning of emotional responses. The numerous studies by psychopharmacologists and comparative psychologists concerning the effects of the new psychotropic drugs on experimentally conditioned emotional responses of animals to previously neutral stimuli have recently been reviewed by Brady[14] and Wikler.[127] In general, the results show that the tranquilizers tend to reduce emotional behavior, while the antidepressant and stimulant drugs tend to increase it. But here again the picture is by no means clear; for powerful compounds such as reserpine and the phenothiazines effectively depress the perform-

ance of instrumental responses that serve to avoid shock, while apparently affecting relatively little the unconditioned components of the response. Less potent compounds such as benactyzine and meprobamate, on the other hand, have little or no effect on the instrumental responses, but seem to alter the unconditioned responses. These differences are still to be understood.

Nevertheless, the study of human subjects in both classical and instrumental conditioning in emotional situations promises to increase greatly the understanding of how psychotropic drugs affect behavior. This approach has been little used. W. H. Gantt and his associates at Johns Hopkins have for more than 20 years conducted many experiments in conditioning responses to emotional stimuli, which Gantt[50] summarized in 1950. New reports appear frequently from their laboratory; but, unfortunately, these workers have not reported studies on the new psychotropic drugs in their experimental situation. Some work bearing on our topic has shown that conditioned responses may be altered by the administration of thyroid extract to hypothyroid patients,[51] by electroconvulsive shock therapy,[46] and by intensive group psychotherapy.[22]

In a study concerning the effects of a tranquilizer on human conditioned responses, Mitchell[94] reported that a 30 day course of chlorpromazine therapy very significantly depressed the conditioning of a GSR in schizophrenic patients, as compared with that in a control group of patients who received no chlorpromazine. These results may be interpreted in the light of Runquist and Ross's[109] recent findings that normal subjects who showed a high degree of emotional responsiveness to noxious stimuli could be conditioned more rapidly than could subjects who showed a lower degree of emotionality. However, Mitchell's results cannot safely be taken to indicate a reduction in anxiety in the schizophrenic subjects, since Taylor and Spence[119] found that both anxiety-neurotic patients and those with other types of neurosis were conditioned more slowly than were psychotic subjects. That the galvanic skin response of schizophrenics may not reflect a heightened anxiety is suggested by an experiment of Howe[70] which showed that schizophrenic individuals had a significantly higher basal skin resistance than did normal subjects, who, in turn, had significantly higher skin resistance than did patients with anxiety. Further, after eight conditioning trials, schizophrenics extinguished the GSR more rapidly than did anxious patients. Mitchell's findings, therefore, may reflect no more than an improved adaptation of his psychotic subjects to the testing situation—an adaptation which made these patients more like normal subjects and thus more refractory to conditioning. This interpretation is supported by an experiment by Truax[120] who found that in normal subjects chlorpromazine actually facilitated acquisition of a conditioned eye-blink response.

In summary, the potentially profitable study of emotional conditioning in humans is practically unexploited. Studies by Mitchell and by Howe cited above yielded superficially conflicting results; chlorpromazine in the one had a depressing effect on the conditioning, while in the other it had a facilitating effect. One study, however, used schizophrenic and the other, normal subjects; one used a GSR response and the other, an eye-blink response; and one used chronic administration of chlorpromazine while the other used acute administration. At this point there is no basis for determining which factor or factors accounted for the disparate findings.

III. Drugs and cognition

A. Extroversion, introversion, and Eysenck's theories. If the studies reported in the preceding section on drugs and emotions have established any point beyond dispute, it is the necessity for an adequate psychophysiologic theory of emotion. This theory of emotion, however, will also have to deal with cognitive processes since the transactional relationships between thinking and feeling are far too obvious to need em-

phasis. At present we must reconcile ourselves to dealing with a variety of theories, each emphasizing a different area of function. Later, as these theories become more refined, they may reveal more general propositions.

In the section to follow, we will deal with some of these alternate areas of emphasis. A particularly good example is found in the work of Eysenck and his co-workers.[42,43,44] In the preceding section we have looked at changes in the acquisition and performance of conditioned responses as evidence of changes in emotionality or motivation. Such changes can also be looked upon as evidence of more general changes in the processes of learning and forgetting.

Eysenck starts from a fundamentally Pavlovian point of view and postulates competing cortical excitatory and inhibitory potentials. These he then relates to extroversion and introversion and in turn to the effects of stimulant and depressant drugs.

He considers that there are competing cortical excitatory and inhibitory potentials. The excitatory potential or factor is responsible for such phenomena as learning and conditioning. The inhibitory factor accounts for extinction of conditioning and is somewhat analogous to the reactive inhibition of Hull (i.e., the inhibition which builds up following a response). Hysteria, brain damage, and depressant drugs are all supposed to increase cortical inhibition and lead to extroversion; while dysthymia (a syndrome characterized by anxiety, tension, rumination, and obsessive thoughts) and excitant drugs decrease cortical inhibition and lead to introversion.

Eysenck[42] writes, "At the test level, we would predict that any test which has been shown to differentiate reliably and validly between introverts and extroverts will, when applied to subjects who have been administered a stimulant (or depressant) drug, show shift in scores in the direction characteristic of greater introversion (or extraversion)," (p. 124). For example, work decrements under conditions of continuous exertion represent the effect of inhibitory potentials building up. Depressants, by increasing cortical inhibition, should increase work decrements and naturally stimulants should have the opposite effect. As might be expected from common experience, these particular effects are found.[43] Under control conditions, however, introverted subjects were found to behave like those treated with Dexedrine, while extroverted subjects tended to behave like those treated with amobarbital.

Eysenck and his co-workers also predict that stimulant drugs will increase the duration of visual aftereffects. This prediction they base on reports by others that extroversion, hysteria, and brain damage go with shortened visual aftereffects. In studies on drugs they do in fact report that stimulants increase the duration of visual aftereffects. Depressants have the opposite effect,[44] and so they argue that visual aftereffects are the result of an excitatory potential.

Eysenck's theories are of great heuristic value but like most current behavioral theories they are intellectual stepping stones rather than finished theoretical structures. For example, on the Stroop test Amytal slowed the test performance while methamphetamine improved the performance. According to Eysenck's theory, this test should then correlate with introversion and extroversion as measured by standard questionnaire items; yet in one experiment[26] an insignificant correlation in the opposite direction was found. Thus a test which was sensitive to drug effects did not correlate in the predicted direction with measures of introversion and extroversion. Brengleman[18] used a figure construction task which correlated with measures of extroversion and introversion. But when subjects were tested against the effect of drugs (Amytal and amphetamine), results in the opposite direction to that predicted by Eysenck's theory were found. Despite such shortcomings, however, Eysenck's theories serve a very valuable purpose by suggesting experiments and stimulating pharmacologic investigators to improve their framework for ordering their findings.

B. Data processing and Bills' blocks. The human mind may be seen as psychotic or nonpsychotic, as the site of the body-image, as an organ intimately concerned with emotion, as a system which reflects cortical inhibition or excitation with extroversion or introversion, or as a data-processing computer. In this last section, we will see how viewing the mind as a data-processing computer can be used in the study of psychopharmacology.

The most recent elaboration of a data-processing model has been by Broadbent.[21] If the human mind usually receives more sensory data than can process, then some filtering and coding processes must be used to reduce sensory input to a usable level. Broadbent presents a model which has the required filtering and coding mechanisms and which seems to explain a wide variety of experimental data. Although he has little to say about psychopharmacology, and although psychopharmacologists have not had much time to make use of his work, this model seems relevant to our problems.

For example, fatigue and boredom have obvious behavioral effects but they often have little or no influence on the performance, particularly if the bored or fatigued subject is allowed to set his own pace. If the experimenter sets the pace of the task and if relevant data are available for less than 1 second at a time, then dramatic decrements in performance do appear. This discontinuous decompensation was first described by Bills[8] and the lapses or gaps in performance that occur with fatigue have been aptly called "Bills' blocks." Broadbent, as quoted by Maag,[20] nicely sums up the situation: "When a man has been working for some time his performance becomes irregular rather than breaking down completely. Crudely speaking, a man is not like a child's mechanical toy which goes slower as it runs down nor is he like a car engine which continues normally until its fuel is exhausted and then stops dead. He is like a motor, which after much use misfires, runs normally for awhile, then falters again and so on."

In his book, Broadbent suggests that one data reducing mechanism in the human is a selective filter. This hypothetical filter will select novel or intrusive stimuli and requires about 1 second to shift away from some routine input to sample a novel input and then go back to the routine input again. If the data relevant to the task are boring or monotonous and if they may be missed when the filter shifts away for a second, then gaps or lapses in performance will appear. If, on the other hand, the subject paces his own task, he will select task-relevant data in the interval between these gaps or lapses and compensate for them by increased spurts of activity.

One of the curious things about sleep deprivation is that sleep-deprived subjects show obvious behavioral effects but may show little decrement in psychological test performance. Williams and his co-workers[125] have been able to demonstrate that if experimenter-paced tasks are presented to sleep-deprived subjects, these subjects show decrements in performance suggesting that in this situation, also, gaps or lapses in attention play a crucial role. Applying these concepts to the field of psychopharmacology, Mirsky and associates[93] find that both phenobarbital and chlorpromazine cause decrements in the performance of the so-called continuous performance task. Their observations seem to indicate that both of these drugs increase gaps or lapses produced by shifting of the selective filter.

The influence of drugs on the intake of sensory data has been considered another way by Callaway and co-workers.[26-29] They find that some drugs make people less responsive than usual to things occurring at the periphery of their attention. Drugs with this psychological effect also seem capable of increasing electroencephalographic arousal. Atropine, which can block or decrease electroencephalographic arousal, seems to have the opposite effect. For example, atropine-treated subjects were more than ordinarily susceptible to interference from peripheral stimuli, yet performed more efficiently than did control subjects when

apparently irrelevant stimuli actually served a useful function. Such findings led these authors to hypothesize that EEG arousal is correlated with a narrowed focus of attention, and a variety of supporting psychological and physiologic data have been assembled in the papers referred to.

But these findings can also be reinterpreted in the light of Broadbent's model. For example, Broadbent's model places a system of limited channel capacity between the selective filter and the effector and long-term memory portions of the central nervous system. Although the selective filter is sensitive to novel stimuli, the limited channel capacity system tends to pass highly probable or likely stimuli much more rapidly than novel stimuli. The properties of both these systems depend on internal probabilistic coding in the central nervous system; that is to say, the intake of sensory data that is influenced by some sort of weighted expectancy. Some stimuli are considered a priori more likely than others.

Intuitively, however, it seems that the aroused individual may relinquish some of this probabilistic coding. Subjectively this would correspond to the feeling of "what now" or "anything may happen." Probabilistic coding tends to reduce information load since, if a series of events have unequal probabilities, they convey less information than does the same series of events with all events equally probable. Thus, if he relinquishes his probabilistic coding, the aroused subject must then resort to filtering and other coding devices to compensate for increased information load. Some of the drug effects previously described as narrowed attention may simply reflect such an altered probabilistic coding.

Such a hypothesis would, for example, fit the data reported by Brengleman.[18] His subjects were required after medication with amphetamine, amobarbital, and a placebo to reproduce figures made up of several geometrical forms or recognize them at the end of ten presentations. Under these particular experimental conditions, recall was relatively easy, and correct responses were the rule. Thus they were considered by the subject as more likely than incorrect responses. Amphetamine is an arousal-producing drug and, according to the hypothesis, should increase the subjective probability of unlikely events, that is, it should make subjects suspect that relatively rare incorrect responses had an increased likelihood of occurrence. Accordingly the subjects should suspect that the number of incorrect responses had been increased following amphetamine; and in fact Brengleman reported that this drug decreased the certainty about adequacy of recall and did so without influencing adequacy of recall itself. Opposite effects were found after the administration of amobarbital, which can be considered an arousal-inhibiting drug.

This probabilistic coding hypothesis can also be related to the previously mentioned work on lapses or gaps produced by the hypothesized selective filter. It will be recalled that this selective filter is particularly sensitive to unlikely events. The more novel the stimulus seems, the more likely it is to capture the selective filter. As a subject becomes bored, fatigued, or sleepy, he presumably becomes less and less aroused. According to the hypothesis, he would then use greater and greater probabilistic coding and because of this, the routine or monotonous task-relevant data would become more and more highly expected, while extraneous stimuli would become more and more subjectively improbable. As these extraneous stimuli (such as proprioceptive sensation and room noise) become more and more novel, they would become more and more likely to capture the selective filter and thus produce the gaps or lapses in performance. An arousal-producing drug like methamphetamine should (and in fact does) reduce the number of performance gaps in fatigued subjects.

Although this theoretical formulation linking change in behavior to arousal has been useful, evidence is now available that it is theoretically inadequate to characterize drugs or procedures simply on the basis of their arousal-producing qualities. For ex-

ample, we usually think of both the heart rate and the galvanic skin response (GSR) as indicators of arousal. In an ingenious experiment Lacey[82] showed that the behavioral correlates of arousal may be quite opposite, depending on which autonomic index of arousal is selected. In his experiments the subject is instructed to raise his hand from a telegraph key as quickly as possible when, following a preparatory signal, a white light in the center of the visual field is momentarily flashed. When any one of six other lights placed peripherally to the right and left of the center light is flashed, the subject is not to react. The longer the run of central lights before a peripheral light comes on, the more likely is a false response to a peripheral light. To Lacey, the false responses to the peripheral light indicate an excitatory phenomenon. But they could equally well be interpreted to indicate an increase in probabilistic coding (with the subject assuming after a long run of central lights that the peripheral light is so unlikely as to make a response to any light correct). According to the probabilistic coding theory, arousal should go with a decreased probabilistic coding and should make the subject behave as though he had seen a shorter series of central lights preceding a peripheral light. He would therefore be less likely to make false responses to a peripheral light. Using the heart rate as an index of arousal, these workers did indeed find that false responses were less likely during periods of high cardiac rate. According to a general theory of alertness or arousal, an increased GSR should also result in diminished incidence of false responses. Lacey's data, however, showed the opposite effect: GSR activity was correlated with an increased probability of false responses.

These experiments thus indicate that, if one correlates arousal with performance, the results will depend on what index of arousal is chosen. It also follows that trying to relate physiologic changes produced by drugs to drug-induced behavioral changes on the basis of an oversimple concept of arousal will inevitably lead into difficulties.

Conclusions

If there is any conclusion to be drawn from this short survey of psychopharmacologic research, it is that no single satisfactory theory fits all the available empiric data. Yet there is no reason for discouragement. Since the behavioral scientist often overestimates the difficulty of his particular field in comparsion with other areas of investigation, the following comment on scientific methodology seems to be apropos: "In the end, what is hoped for is a theory to incorporate all the phenomena involved. Nonetheless it is possible to start writing down theories with different areas of emphasis and as each is refined they should tend to reveal the common truth. This is a situation not solely characteristic of . . . ." We would end this sentence with the phrase, "the theory of psychopharmacology," instead of the particular author's conclusion, ". . . the theory of superconductivity."[83] We behavioral scientists may also reconcile ourselves, at least for the time being, to a variety of theories, each with various areas of emphasis. Yet we may also hope that some day more unifying theories will be discovered. In the meantime while developing these isolated theories, we should observe certain cautions.

We must keep continually in mind that psychopharmacology deals with behavior. No psychopharmacologic theory is better than its weakest link, and this weakest link is very likely to be the behavioral link. As we have seen in this field, there is a tendency to gloss over the psychological and behavioral side of a psychopharmacologic theory by using poorly defined behavioral and psychological terms, while lavishing all the scientific controls on the biochemical and physiologic side of the study. This tendency is what Benjamin[5] has referred to as "psychophobia." It seems that, like other phobias, psychophobia is also a defense mechanism. The business of science (and the pleasure of scientists) is to build models and construct theories that make observations assume some order and closure. The rather unsatisfactory characteristic of most

psychological theories that have been used in psychopharmacology has been pointed out. This unsatisfactory and vague character may play a role in evoking psychophobia, since the physicochemical statements seem at least superficially to be much more tidy. Unsettling as the fact may be, the psychological aspects of drug actions claim an important part of our attention and demand the most sophisticated approaches available.

This plea for greater psychological sophistication, however, in no way exonerates the sophisticated behavioral scientist from the charge of physiologic naïveté. There is a strong tendency toward oversimplification of the underlying physiologic phenomena by the behavioral scientist. For example, he may consider the reticular formation as having a unitary physiologic function, and arousal as some absolute state of the central nervous system which can be defined by some universally invariant changes in peripheral autonomic variables. Unfortunately, life is not that simple.

In spite of all these difficulties, we can see that the new psychotropic drugs apparently alter clinical ratings and descriptions of behavior. These observations suggest generalizations about drug effects; but when specific and objective measures are used many contradictory findings emerge. The clarification of these contradictions demands a more complex yet integrated theory of behavior. The new psychotropic drugs can serve as an invaluable class of independent variables to aid in the development of such theories.

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Book reviews

Clinical Evaluation of New Drugs, S. O. Waife and A. P. Shapiro, editors. New York, 1959, Hoeber-Harper, Inc. 223 pages.

Quantitative Methods in Human Pharmacology and Therapeutics, D. R. Laurence, editor. London, 1959, Pergamon Press, Inc. 253 pages.

These two multi-authored monographs on the problems of drug evaluation in man speak volumes (pun intended) for the great interest as well as the need for information on how to establish that a drug produces an effect in man, how to measure how much effect it produces, and how to determine whether it is useful as a therapeutic agent. Although the articles in the two books explore the same subject matter, and it would therefore seem that a considerable amount of duplication would be found, it is surprising that they are completely different and make useful companion pieces.

The work edited by Waife and Shapiro is a collection of fifteen articles, mostly on the general aspects of clinical drug evaluation, written in a very readable, if not popular, style. Articles such as, "Statement of the Problem," "From Animals to Man," "The Training of the Investigator," and "The Investigator Himself," comprise the larger part of the volume and give it its over-all tone, which is philosophic. Even though the second part is entitled, "Clinical Trials in Practice," it gives little of the specific and technical information an investigator would need in order to design a clinical pharmacologic study for himself.

Because of its general approach the book provides the information the noninvestigator should have about clinical evaluations without subjecting him to the technical details on methodology he may not be interested in. While using specific examples to illustrate general points and proving that the book deals with real, and not imaginary problems, it provides the reader with the basic approach he needs for critical reading on clinical evaluation. This seems to have been the objective of the editors and the book successfully attains it for them.

The volume edited by Laurence is an account of the proceedings of an outstanding symposium on methodology in human pharmacology and therapeutics. Its target is the investigator, although here too there are some introductory articles of general

interest by J. H. Gaddum and H. Gold and, by way of conclusion, one by T. T. Fox. The remaining articles are devoted fairly strictly to the details of specific methodology and exactly how to obtain results of some precision and reliability in the evaluation of drugs in man. There are therefore articles on the mensuration of such biologic reactions as anesthesia, analgesia, neuromuscular blockade, and toxic effects. The techniques described in the volume are merely samples from a large field, and much is untouched. However, whatever is presented is well done. The articles are written in the standard formal style of the scientific article, tersely and clearly, but are livened by excerpts from the discussion which followed the papers when they were delivered. This is clearly a book for the clinical investigator; he *should* have it.

Neither book is complete in itself, nor does the combination provide a complete text on the subject, but between them they bring together information which until now has been widely scattered in the literature. They certainly provide more comprehensive handling between hard covers than this important subject has heretofore received.

Walter Modell, M.D.

Sensitivity Reactions to Drugs: A symposium organized by the Council for International Organizations of Medical Sciences, M. L. Rosenheim and R. Moulton, editors. Springfield, Ill., 1958, Charles C Thomas, Publisher, and Oxford, England, Blackwell Scientific Publications.

In July, 1957, a symposium on "sensitivity reactions to drugs" was held in Liège, Belgium. Professor M. L. Rosenheim served as chairman of this symposium which was made possible by cooperation of the Council for International Organizations of Medical Sciences with one of its member-organizations, namely, the International Society of Clinical Pathology. The present volume contains the papers presented at this symposium, enriched by the transcript of the discussion in which a great many otherwise unpublished experiences of participants were communicated. Discussions usually reveal more about the highly personal manner in which an investigator approaches his field than formal and polished papers do. Accordingly, it is refreshing to read in these discussions the expression of beliefs, idiosyncrasies of opinions and prejudices which, in general, we have learned to conceal carefully in order not to offend editorial boards of professional journals.

It gives testimony to the excellent planning of the symposium that the chairman clarifies, in the introduction to the monograph, the terminology regarding "unwanted effects of drugs." In this, Professor Rosenheim follows largely the suggestions made by E. A. Brown in 1955. The pharmacologically minded reader may be reluctant to accept the designation of "specific side effects" for manifestations which must be attributed either to physiologic and biochemical mechanisms set into play by the primary drug action, or to drug actions at more than one effector site. Such instances , however, in the present symposium, only touched upon, whereas the "hypersensitivity-allergic reactions" constitute the primary subject of the conference.

The multitude of different aspects of and approaches to "sensitivity reactions to drugs" probably equals the number of disciplines in basic and clinical medical science. This belief is supported by the impressive variety of methods of inquiry into the occurrence and nature of hypersensitivity-allergic reactions as presented in this symposium: It ranges from a general review on hemolytic reactions to drugs (G. Discombe) and from a casuistics of megaloblastic anemia in patients receiving anticonvulsant drugs (J. N. Marshall Chalmers) to attempts to reach a decision about the distinctiveness of delayed-type and immediate-type hypersensitivities (Merrill W. Chase). J. F. Ackroyd reviews extensively clinical aspects and immunologic basis of

two types of mechanisms which lead to thrombocytopenic purpura. As he had shown previously, occurrence of the Sedormid-type (platelet lysis only) or of the antazoline type (lysis *and* immune precipitate formation) is not so much determined by the drug used as by the idiosyncracy of the patient. J. Dausset adds valuable information to the concept of the "periplatelet atmosphere" which appears to constitute an envelope of some special protein; to this, antigenic complex formation with Sedormid is convincingly attributed. The possible role of autoantibody production as mechanism responsible for agranulocytosis due to drug sensitivity is discussed by S. Moeschlin. A unifying concept is thereby proposed which places agranulocytosis precipitated by drug sensitivity in the same category as leukopenia of some virus infections, infectious mononucleosis, some chronic bacterial infections, and that associated with some neoplastic diseases; the common denominator in all these cases would be the appearance in the organism of an abnormal product that may lead to autoantibody formation. Tissue reactions produced by sensitivity to drugs, including the so-called collagen diseases and arteritis, are discussed in well-illustrated contributions of A. R. Rich, W. St. C. Symmers and L. Meyler. One may wonder why so little was said about sensitivity reactions to antibiotics; only the bone marrow aplasia caused by chloramphenicol is specifically discussed.

Chemical and pharmacologic considerations relating to the problem of drug sensitivity are reviewed by G. E. Davies in a most stimulating, provocative, and imaginative way. In particular, attention is directed to the possible role of drug metabolites and the reactive groups on proteins with which they might combine in such a way that the complex is antigenic. If reference is made to the classical contributions in this field—as it is in Davies' paper—then I think the record should be set straight by pointing out that Obermeyer and Pick opened up this whole field of study in 1906. Their work indicated for the first time that it would be possible to introduce into proteins new chemical structures which would determine their antigenic properties.

The diversity of subjects covered in the symposium, as well as the uniformly high standard of presentations and the excellency of illustrations, makes this publication a rich and pleasant source of information.

Gerhard Werner

Announcement

Negative reports

At the meeting of the Editorial Board of CLINICAL PHARMACOLOGY AND THERAPEUTICS on Dec. 5, 1959, it was decided that, because of their archival value and in order to prevent needless duplication of clinical investigations, concise reports of negative results with new drugs would be acceptable for publication in this journal. Although these contributions must be brief, they should nevertheless indicate both background and method used and should include a useful bibliography.